"ASSOCIATION OF LIPOPROTEIN (A) AND CAROTID INTIMA MEDIATHICKNESS IN RHEUMATOID ARTHRITIS PATIENTS IN PREDICTION OF CARDIOVASCULAR RISK"

Dissertation Submitted to

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

In partial fulfilment of the requirements

For the award of degree of

M.D. (Branch-XIII)

BIOCHEMISTRY

GOVERNMENT STANLEY MEDICAL COLLEGE & HOSPITAL

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY, CHENNAI, TAMILNADU

MAY - 2019

CERTIFICATE

This is to certify that the dissertation titled,**"Association ofLipoprotein(a) And Carotid Intima Media Thickness in Rheumatoid Arthritispatients Inprediction ofCardiovascular risk"**is a genuinework doneby **Dr. Lakshmi.S.V**.for the partial fulfillmentof the requirements forM.D(Biochemistry) Branch XIII Examination of The Tamil Nadu Dr. M.G.R. MedicalUniversity to be held in May 2019,under the Supervisionof **Dr. R.SHANTHI, M.D**during the academicperiod 2016-2019.

Dean
Stanley Medical College
Chennai-1

Professor & HOD
Dept. Of. Biochemistry
Stanley Medical College
Chennai-1

CERTIFICATE BY GUIDE

This is to certify that the dissertation on **"Association of Lipoprotein (a) and Carotid Intima Media Thickness in Rheumatoid Arthritis patients in prediction of Cardiovascular risk"** is a record of research work done by **Dr.S.V.Lakshmi** in partial fulfilment for **M.D (BIOCHEMISTRY)**Examination of The Tamil Nadu Dr.M.G.R.Medical University to be held in May 2019. The period of study is from December 2017 to July 2018.

Dr.M.P.SARAVANAN,M.D
Professor, Department of Biochemistry,
Government Stanley Medical College,
Chennai – 600001.

DECLARATION

I, **Dr.S.V.Lakshmi,** solemnly declare that the dissertation titled**"Association of Lipoprotein (a) and Carotid Intima Media Thickness in Rheumatoid Arthritis patients in prediction of Cardiovascular risk"** is a bonafidework done by me during the period of DECEMBER 2016 to JULY 2017 at Government Stanley Medical College and Hospital, Chennai under the expert guidance of

Dr.M.P.SARAVANAN,M.D

Professor, Department of Biochemistry,
Government Stanley Medical College and Hospital,
Chennai-600001.

This thesis is submitted to The Tamil Nadu Dr. M.G.R. Medical University in partial fulfillment of the rules and regulations for the M.D. degree examinations in Biochemistry to be held in May 2019.

Chennai-1 **Dr.S.V.Lakshmi**

Date:

GOVERNMENT STANLEY MEDICAL COLLEGE& HOSPITAL,CHENNAI -01

INSTITUTIONAL ETHICS COMMITTEE

Title of the Work : "ASSOCIATION OF LIPOPROTIEN (A) AND CAROTID INTIMA MEDIA THICKNESS IN RHEUMATOID ARTHRITIS PATIENTS IN PREDICTION OF CARDIOVASCULAR RISK".

Principal Investigator : DR. S.V.LAKSHMI,

Designation : M.D BIOCHEMISTRY II YEAR,

Department : Department of **BIOCHEMISTRY**, Govt. Stanley Medical College.

The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on 21.12.2017 at the Council Hall, Stanley Medical College, Chennai-1 at 10am.

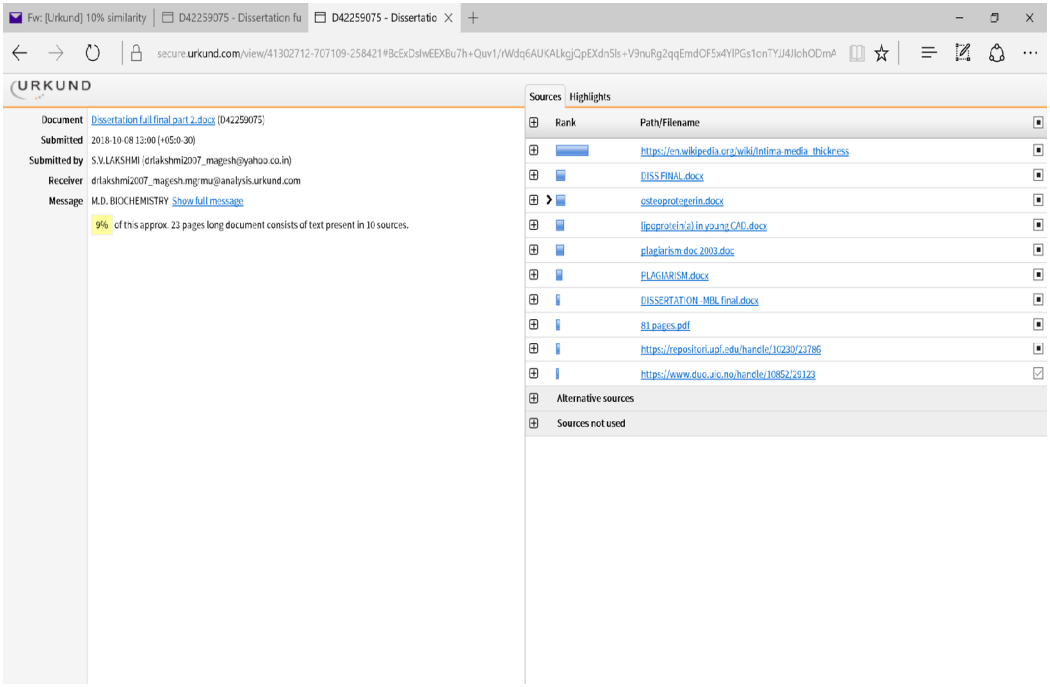
The members of the Committee, the secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

The Principal investigator and their team are directed to adhere to the guidelines given below:

1. You should inform the IEC in case of changes in study procedure, site investigator investigation or guide or any other changes.
2. You should not deviate from the area of the work for which you applied for ethical clearance.
3. You should inform the IEC immediately, in case of any adverse events or serious adverse reaction.
4. You should abide to the rules and regulation of the institution(s).
5. You should complete the work within the specified period and if any extension of time is required, you should apply for permission again and do the work.
6. You should submit the summary of the work to the ethical committee on completion of the work.

31/1/18

MEMBER SECRETARY,
IEC, SMC, CHENNAI


Fw: [Urkund] 10% similarity
D42259075 - Dissertation fu
D42259075 - Dissertatio
secure.urkund.com/view/41302712-707109-258421#BcExDslwEEXBu7h+Quv1/rWdq6AUKALkgjQpEXdn5ls+V9nuRg2qqEmdOF5x4YlPGs1cnTYJ4JlchODmA
URKUND
Document Dissertation full final part 2.docx [D42259075]
Submitted 2018-10-08 13:00 (+05:0-30)
Submitted by S.V.LAKSHMI (drlakshmi2007_magesh@yahoo.co.in)
Receiver drlakshmi2007_magesh.mgrmu@analysis.urkund.com
Message M.D. BIOCHEMISTRY Show full message
9% of this approx. 23 pages long document consists of text present in 10 sources.
Sources
Highlights
Rank
Path/Filename
https://en.wikipedia.org/wiki/Intima-media_thickness
DISS FINAL.docx
osteoprotegerin.docx
lipoprotein(a) in young CAD.docx
plagiarism doc 2003.doc
PLAGIARISM.docx
DISSERTATION -MBL final.docx
81 pages.pdf
https://repositori.upf.edu/handle/10230/23786
https://www.duo.uio.no/handle/10852/29123
Alternative sources
Sources not used

CERTIFICATE - II

This is to certify that this dissertation work titled **"Association of Lipoprotein (a) and Carotid Intima Media Thickness in Rheumatoid Arthritis patients in prediction of Cardiovascular risk"** of the candidate **Dr.S.V.Lakshmi,** post graduate in Biochemistry with registration Number **201623052** for the award of **M.D.BIOCHEMISTRY** in the **Branch XIII.** I personally found that the uploaded thesis file contains from introduction to conclusion pages and result shows **9%** (Nine percentage) of plagiarism in the dissertation

Guide & Supervisor sign with seal

ACKNOWLEDGEMENTS

First and foremost, I thank the Almighty. Without Him, nothingwould been possible. With God's Grace, everything is possible.

I wish to express my sincere thanks to **Prof. Dr. S. PONNAMBALA NAMASIVAYAM, M.D.,DNB.,DA.,** Dean Government Stanley Medical College and Hospital for having permitted me to utilize the facilities of the hospital for the conduct of the study.

I sincerely thank our **Prof& HOD,Dr.R.SHANTHI, M.D.,D.C.P.,** Professor and Head, Department of Biochemistry, Government Stanley Medical College and Hospital for her inspiration from the beginning of the course with valuable suggestions, expert supervision and support.

My heartfelt gratitude to **Prof. Dr. M.P. SARAVANAN, M.D.,** for his motivation, kindness, valuable suggestions, expert supervision, valuable guidance and support right from my presentation in ethical committee which enabled me to complete this study.

I thank **Associate Prof. Dr. M. VIJAYALAKSHMI, M.D.,** for her ideas and immense support, helpful suggestions and corrective comments.

I thank **Associate Prof. Dr.V.G.KARPAGHAVALLI, M.D., D.G.O.,** for her constant encouragement and support.

Ithank Prof. **Dr.C.AMARNATH, M.D.,DNB.,FRCR.,PhD** Professor and Head of the Department of Radiology, for permitting me to do the study in radiology department and also giving valuable suggestions regarding my study protocol.

I thank **Dr.M.HEMAM.D.,** Asst. Professor, Department of Rheumatology, for permitting me to conduct the study in Rheumatology OPD andalso for giving valuable suggestions regarding my study protocol and corrective comments.

I thank **Dr.P.Seenivasan M.D.,**Professor and HOD of community Medicine and **Dr.T.Susila M.D.,** Asst.Professor, Department of community Medicine for permitting To collect samples at master health check up and guiding me in the Biostatistics.

I wish to thank all Assistant Professors especially for their aid, keen interest, encouragement and corrective comments during the research period.

I wish to thank all Associate Professors and post graduates in department of Radiology, who helped me to do carotid doppler for my study groups dedicatedly without any hesitation during the research period.

Heartfelt thanks are due to my postgraduate colleagues, for never hesitating to lend a helping hand for me throughout the study.

I thank all the staffs and technicians, Government Stanley Medical College and Hospital for their cooperation and assistance.

I owe my gratitude to all the patients included in the study and their relatives, for their whole hearted co-operation and consent.

I am indebted to my family members for their care and moral support during the period of my study, without which it would be difficult for me to complete this study.

I thank my husband Mr.K.Magesh.M.B.A. for the care and moral support during the period of my study, without him it would be difficult for me to complete this study. I thank to my son K.M.Anbuchelvan for amoral support during the study period.

Last but not the least; I would like to thank all the patients who cooperated with me throughout my work.

CONTENTS

ABBREVIATIONS

RA : Rheumatoid Arthritis

Lp(a) : Lipoprotein (a)

c/C IMT : Carotid Intima Media Thickness

TC: Total Cholesterol

TGL: Triglyceride

LDL: Low Density Lipoprotein

VLDL: Very Low Density Lipoprotein

HDL: High Density Lipoprotein

CM: Chylomicron

ANA: Anti Nuclear Antibody

CVD: Cardio Vascular Disease

IL: Interleukin

GWAS: Genome Wide Association Studies

MHC: Major Histocompatibility Complex

SE: Shared Epitopes

HLA-DRB: Human Leucocyte Antigen (HLA), haplotypes

SNPs: Single Nucleotide Polymorphisms

PTPN 22: Protein Tyrosine Phosphatase Non-receptor 22

TNF-α: Tumor necrosis Factor-α

EBV: Epstein Barr Virus

Anti-CCP: Anti- Cyclic Citrullinated Peptides Antibody

APCs: Antigen Presenting Cells

RFs: Rheumatoid Factor

GM-CSF: Granulocyte – Macrophage Colony Stimulating Factor

Pre OC: Osteoclast Precursors

RANK: Receptor Activator of Nuclear Factor

RANK-L: Receptor Activator of Nuclear Factor

ACPA: Anti Citrullinated Peptide Antibodies

CCP: Cyclic Citrullinated Peptide

CRP: C-Reactive Protein

ESR: Erythrocyte Sedimentation Rate

IP: Interphalangeal joint

MCP: Metacarpophalangeal joint

MTP: Metatarsophalangeal joint

PIP: Proximal Inter Phalangeal joint

ULN: Upper limit of normal

MRI: Magnetic Resonance Imaging

EPCs: Endothelial Progenitor Cells

AI_X: Augmentation Index

LPS: Lipoprotein Lipase

HL: Hepatic Lipase

$ABCA_1$: ATP Binding cassette Transporter-1

DM: Diabetes Mellitus

O_X-LDL: Oxidized LDL

DMARD: Disease-modifying antirheumatic drug

CHD: Coronary Heart Disease

Apo(a): Apoprotein (a)

LRP: LDL Receptor Related Protein

INTRODUCTION

Rheumatoid Arthritis(RA) is a chronic, autoimmune, inflammatory disorder of unknown aetiology[1], that affects ~1% of general population[2],0.5% of adult population worldwide[3].It primarily involves smaller and larger synovial joints causing symmetric polyarthritis[1].It may also affect many other organs and tissues including heart, kidneys, lungs, eyes, salivary glands, blood vessels and nerve tissue.

Among all the extra-articular involvement, CardioVascular Disease (CVD) is the leading cause of mortality in RA patients and its accounts for 35%-51% of all mortality in RA patients[4], with a decrease in life expectancy by 3-10 years.[3] Coronary Heart Disease (CHD) risk is greater in patients with RA by approximately 1.5-fold to 2-fold (i.e., relative risk).[5] The excess vascular risk manifests differently in RA patients compared with patients without RA, with less angina, but more sudden deaths and unrecognized myocardial infarctions.[5]

The process of atherosclerosis in RA is a dynamic inflammatory process.[6] This inflammation leads to impaired endothelial dysfunction that causes systemic release of inflammatory mediators like Tumor Necrosis Factor-α (TNF-α),Interleukin-1 (IL) and IL-6.[1] Atherosclerosis begins with endothelial activation, leukocyte recruitment, lipid oxidation, plaque destabilization and thrombosis.[6]

Framingham Heart study established a clear paradigm in which higher Total cholesterol (TC) level is associated with increased cardiovascular risk inGeneralpopulation. But in RA patients a different relationship was observed – that is lower TC is associated with increased cardiovascular risk.[7] Thus an absence of abnormal lipid profile does not rule out the possibility of coronary vascular disease in these patients.

In RA patient lipid profile varies depending on disease activity and duration. In these patients, inflammation causes changes in both increased Lipoprotein(a) [Lp(a)] and decreased High Density Lipoprotein (HDL) leading to dyslipoproteinemia.[1]

Lp(a) is one of the important risk factors for premature atherosclerosis.[1] Several studies show a direct relationship between Lipoprotein(a) and coronary artery disease in general population and Type-2 Diabetes Mellitus (DM).[8] In our study we estimate the relationship between Lp(a) and Dyslipoproteinemia with cardiovascular risk in RA patients.

This subclinical atherosclerosis can demonstrated by increased Carotid artery Intima Media Thickness (CIMT).[6]CIMT is a doppler measurement of carotid artery, that measures the intima, media layers- where the atheroma begins.

It is considered as a good marker of atherosclerosis in its initial stage. Both Carotid plaques and Carotid artery IMT are associated with higher risk of cardiovascular mortality.[9]

The purpose of our present study was to measure Carotid IMT and Carotid plaques by using high resolution B-mode ultrasound and colour doppler to assess the existence of subclinical atherosclerosis in RA patients and healthy controls.[10]

Hence, we find out the correlation between Lp(a) and cIMT and lipid profile in RA patients and control groups.

REVIEW OF LITERATURE

Rheumatoid arthritis (RA):

RA is a chronic inflammatory autoimmune disease of unknown aetiology characterized by a symmetric, peripheral polyarthritis[13]. It is the most common form of chronic inflammatory arthritis and often results in joint damage and physical disability[13]. It is a systemic disease and hence may affect other organs including heart, lungs, eyes and skin.[14]

Compared to men, RA disease develops 2 to 3 times more common in women.[14] The incidence of RA increases between 25 and 55years of age, after which it plateaus until the age of 75 and then decreases[13]. For men symptoms develop later in life.[14]

Aetiology:

The exact aetiology of RA is unknown.[16] The probable risk factors include.

(I) There may be a Genetic basis for development of RA disease[13]

(i)The alleles known to confer the greatest risk of RA are located within the Major HistoCompatibility Complex (MHC)[5]. It has been estimated that one-third of the genetic risk for RA resides within MHC locus. Most but not all of this risk is associated with allelic variation in the HLA-DRB1gene (which encodes the MHC IIβ-chain molecule). The disease-associated HLA-DRB1alleles share an amino acid sequence at positions 70-74in the third hypervariable regions of the HLA-DR β-chain known as Shared Epitope (SE).

Carriership of the SE alleles is associated with production of Anti-Cyclic Citrullinated Peptides Antibody (Anti-CCP) and worse disease outcomes[13].

(ii) Genome-Wide Association Studies (GWAS) are based on the detection of Single-Nucleotide Polymorphisms (SNPs).

(iii)Among the best examples of the non-MHC genes contributing to the risk of RA is gene encoding Protein Tyrosine Phosphatase Non-receptor 22 (PTPN22)[13]

(2)Estrogen plays an important role.[13,16]

(i) Enhances immune response.

(ii)Estrogen can stimulate production of TNF-α, a major cytokine in the pathogenesis of RA.

(3) Environmental factors:

(1) Infectious aetiology:[5,13]

(i) bacterial -Proteus, Enteric bacteria.

(ii)viral infections -Epstein-Barr virus (EBV), Parvovirus-B19

(2)Cigarette smoking increases the risk of the disease in men.

Smoking increasethe risk of RA with HLA-DR4 and act synergistically with HLA-DRB1.[16]Long term exposure to tobacco smoke has capacity toinduce citrullination of cellular proteins in the lung and stimulate the expression of a neoepitope. Neoepitope is capable of inducing self-reactivity and leads to formation of immune complexes and joint inflammation.[13]

(3) Exposure to silicone dust, asbestos, wood and mineral oil has also been linked toan increased for anti-CCP antibody-positive RA.[13]

Pathogenesis of RA:[13]

Genetic predisposition along with environmental factors may trigger the development of RA, with subsequent synovial Tcell activation. CD4+ T cells become activated by Antigen-Presenting Cells (APCs) through interactions between the Tcell receptor and Class II MHC-peptide antigen (signal 1).

Inside the joint ligands binding Toll-like Receptors (TLRs) may further stimulate activation of APCs. Synovial CD4+Tcells differentiate into $T_H1$and T_H17 cells; each with their distinctive cytokine profile.CD4+T_H cells in turn activate B cells. Immune complexes (like Rheumatoid factors (RFs), anti-CCP antibodies) may form inside the joint and activating the complement pathway and amplifying inflammation.

T effector cells stimulate synovial macrophages(M) and fibroblast (FB) to secrete proinflammatory mediators -TNF-α. TNF-α upregulates adhesion molecules on endothelial cells and promoting leukocyte influx into the joint. It also stimulates the production of other inflammatory mediators, like IL-1,IL-6 and Granulocyte-Macrophage Colony-Stimulating Factor(GM-CSF). TNF-α has a critically important function in regulating the balance between bone destruction and formation.

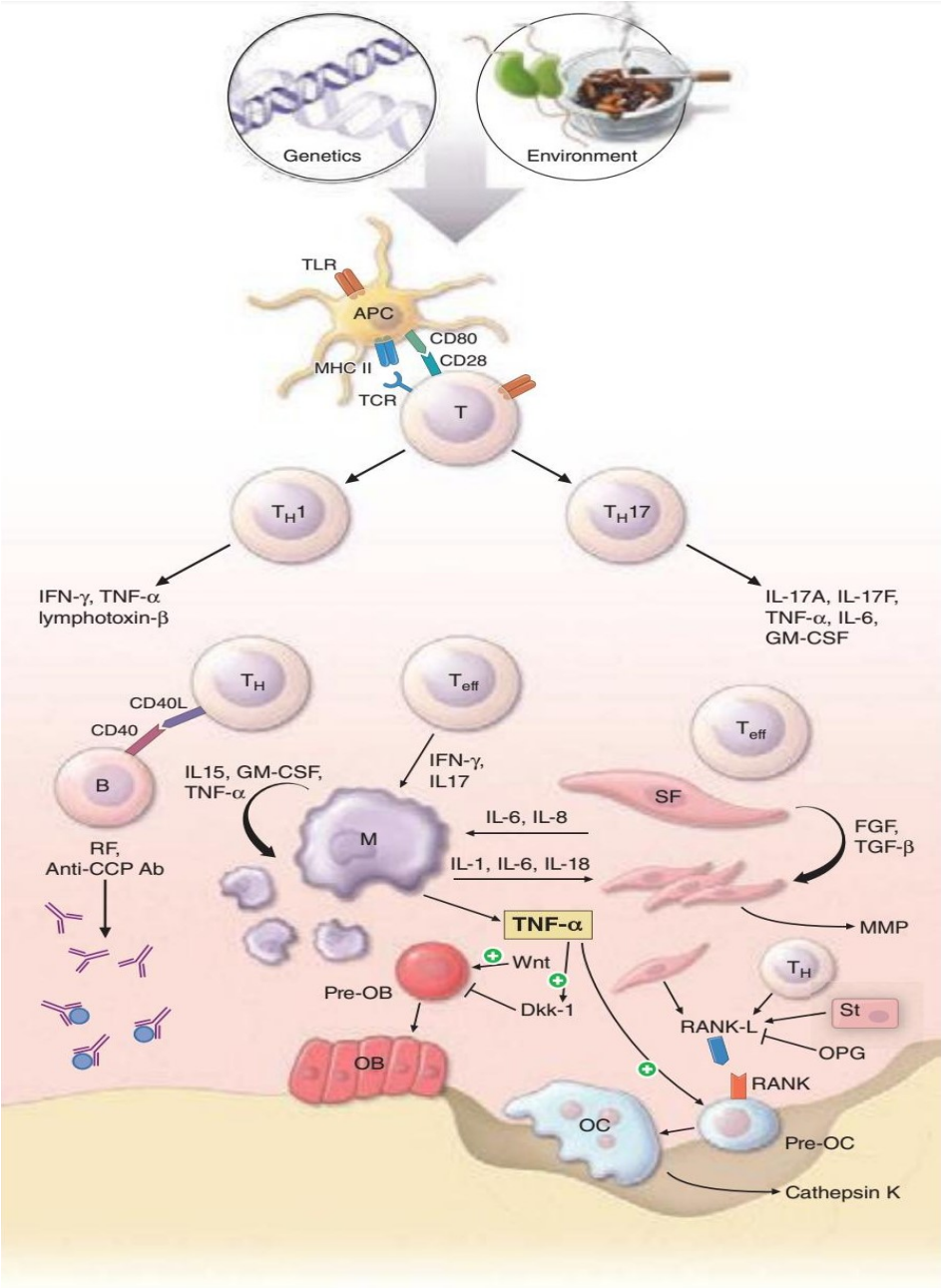


Fig 1: Show pathophysiological mechanisms of RA[13].

TNF-α stimulates osteoclastogenesis. However, it is not sufficient byitself to induce the differentiation of osteoclast precursors (Pre-OC) into activated osteoclasts capable of eroding bone. Osteoclast differentiation requires the presence of colony stimulating factor and Receptor Activator of Nuclear

Factor-Kb(RANK) ligand(RANKL), which binds to RANK on the surface of Pre-OC. Inside the joint, RANKL is mainly derived from stromal cells, synovial fibroblasts, and Tcells. Osteoprotegerinacts as a decoy receptor for RANKL, thereby inhibiting osteoclastogenesis and bone loss.[13]

CLINICAL FEATURES:

Patients often complain of early morning joint stiffness lasting more than 1hr that eases with physical activity. The earliest involved joints are typically the smaller joints of the hands and feet. The initial pattern of joint involvement may bemonoarticular, oligoarticular (≤4 joints), or polyarticular (>5 joints), usually in symmetric distribution.[13]

In RA, hands are most commonly affected- wrists, metacarpophalangeal (MCP), and proximal interphalangeal (PIP) joints are the most frequently involved joints. Flexor tendon tenosynovitis is a common feature of RA and leads to decreased range of motion, reduced grip strength and also “trigger” fingers. Progressive destruction of the joints and soft tissues may lead to chronic, irreversible deformities like “swan neck deformity”, “boutonniere deformity” and “Z-line deformity”. Other joints include elbows, knees, feet, hips, shoulders and atlantoaxial joints of cervical spine.[13,16]

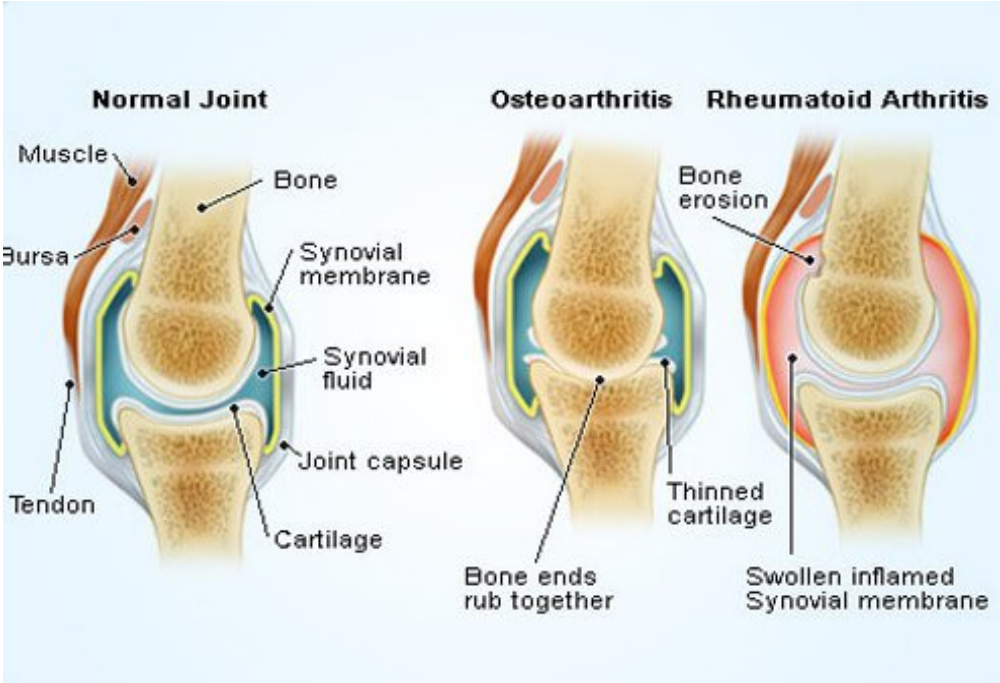


Fig 2: Show comparison of Normal joint, Osteoarthritis & Rheumatoid Arthritis joints.

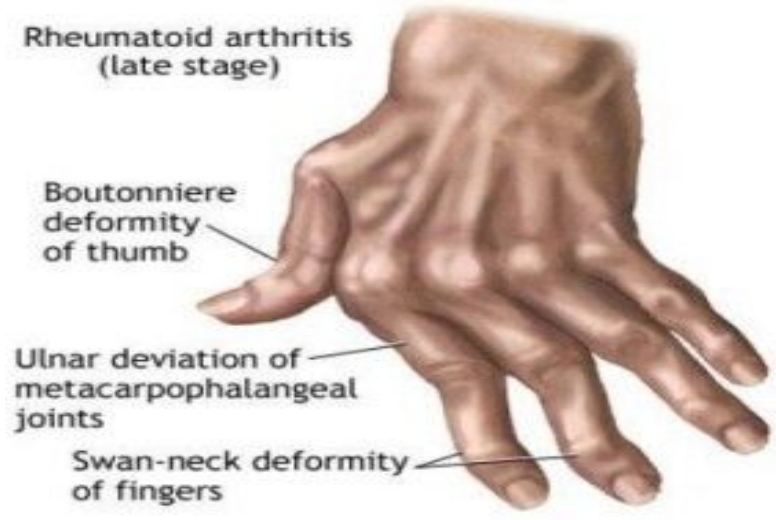


Fig 3: Show Deformities of hand in RA patient.

The following pictures show joint deformities of RA patients who attend the Rheumatology OPD Stanley Hospital during this study period

Fig 6:
Boutonniere deformity of fingers

Fig 7:
Fixed Elbow Deformity

Fig 4 :
Ulnar Deviation of little finger

Fig 5 :
Boutonniere Deformity of Thumb

Fig 8:
Swan neck Deformity of Hands

Fig 9:
Hallux Varus Deformity

Fig: 10
A) Bouchard's node B)Heberden's node

Fig: 11
Comparison of both hands in RA

The most common systemic and extraarticular manifestations of RA are (i)constitutional symptoms include fatigue, loss of appetite, loss of weight, low grade fever, malaise and depression. The other symptoms are (ii) subcutaneous nodules, (iii) scleritis, (iv) pleuritis and (v) pericarditis (vi) increased risk of

atherosclerosis, (vii) vasculitis and (viii)normochromic normocytic anemia- is the most common hematologic abnormality.[13]

RA patients may experience **flareup** of symptoms that can last for days to weeks, followed by periods of **remission.**Remission can last for weeks, month, or years, during which patients have few or no symptoms. Symptoms reappear after a period of remission which is called as **relapse**. The course of illness varies from patient to patient.[5,13]

Investigations:

The following Investigations were done to make a diagnosis of rheumatoid arthritis. [5,13,16]

(i) Nonspecific inflammatory markers: ESR & CRP.

(ii) Rheumatoid factor (RF) – IgM, IgG, IgA isotypes.

(iii) Antinuclear antibodies (ANA).

(iv) Joint imaging: Plain x-ray of Affected joint(s)

(v) Synovial fluid analysis – Synovial fluid White blood cell (WBC) counts can vary widely, but generally range between 5000-50,000WBC/μL compared to <2000WBC/μL for a non-inflammatory condition such as osteoarthritis.

(vi) Ultrasound and magnetic resonance imaging (MRI)for joints.

(vii) Anti-cyclic citrullinated peptide antibodies (ACPA).

The overall mortality rate in RA is two times greater than general population, with ischemic heart disease being the most common cause of death followed by

infection. [1] The other causes of death in RA are lymphoproliferative disorders and gastrointestinal bleeding.[15]

Pathogenesis of atherosclerosis in RA:

There is an evidence that persistent, high-grade inflammation present in RA is the main cause of development of premature atherosclerosis and its complication.[15]The release of number of proinflammatory mediators from inflammation of RA is associated with accelerated atherosclerotic process[17]. These cytokines not only cause the local inflammation and joint destruction, but also affects the vascular system like liver, adipose tissue and vascular endothelium resulting in unfavourable, 'proatherogenic' state.Inflammatory markers of endothelial activation play an important role in cardiovascular risk. Chemokines and Endothelial adhesion molecules are the important factors for the release and extravasation of inflammatory cells into the interstitial matrix.[17]

ClassificationCriteria for Diagnosis of Rheumatoid Arthritis: [13]

Joint involvement	1 large joint (shoulder, elbow, hip, knee, ankle)	**0**
	2-10 large joints	**1**
	1-3 small joints (MCP, PIP, thumb, IP, MTP, wrist)	**2**
	4-10 small joints.	**3**
	>10 joints (at least 1 small joint)	**5**
Serology	Negative RF and negative ACPA	**0**
	Low-positive RF or low-positive anti-CCP antibodies (≤3times ULN)	**2**
	High-positive RF or high-positive anti-CCP antibodies (>3 times ULN)	**3**
Acute-phase reactants	Normal CRP and normal ESR	**0**
	Abnormal CRP or abnormal ESR	**1**
Duration of symptoms	<6weeks	**0**
	≥6weeks	**1**

A **score of ≥ 6**fulfills requirements for **definite RA.**

The prothrombotic factors like “fibrinogen, von Willebrand Factor(vWf) and tissue plasminogen activator antigen(tPa)or D-dimer” are also elevated in these patients.[21]

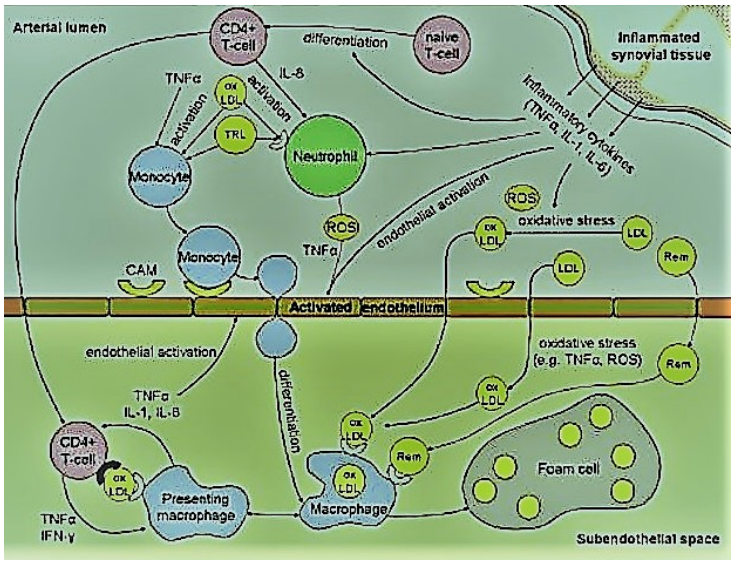


Fig 12: Shows Release of proinflammatory cytokines from the synovial tissue.

(i)Endothelial dysfunction:

In RA patients the endothelium shows the signs of dysfunction. It has been shown that the endothelium-dependent dilatation is impaired in RA patients when compared to control.[23] It is also evident that the impairment of the endothelium is at cellular level. The Endothelial progenitor cells (EPCs) which is secreted by hematopoietic system. This EPCs plays an important role in the development and maintenance of the endothelial cell layer and very effectively repairs the vascular endothelium. This EPCs protect against ischemia and

atherosclerosis.[24] The number of circulating EPCs was found to correlate inversely with the Framingham risk factor score.[27]

In RA patients this EPCs are reduced in number, which is associated with dysfunction of endothelium.[28] This reduced number of EPCs is inversely correlated with disease activity.[29]

(ii) Alterations in vascular function:

In RA patients the function of vascular system is altered. Arterial stiffness is the predictor of mortality and cardiovascular risk.[30]The Pulse wave velocity is the marker of arterial stiffness. In RA patients the pulse wave velocity is higher than control. That reflecting the arterial stiffness is increased in RA patients.[31] The "Augmentation index" (AIx) is another marker for vascular dysfunction.[32] These changes are associated with unfavourable energy requirement of heart during systole. As a result, the ejection into the stiffer vessels is associated with a higher energy requirement. This will cause unfavourable supply/demand relationship of the myocardium.[34,35] In RA not only the vascular dysfunction of macrovascular system, the microcirculation is also affected.[36]

(iii)**Cardiovascular morbidity and mortality:**

In RA patients all these changes are associated with increased cardiovascular morbidity and mortality[22] However, in RA, the cardiovascular risk factors aresimilar to diabetes. In a prospective study, for RA patient and General population, the 3-year incidence rate of fatal and nonfatal cardiovascular events

was 9.0% and 4.3% respectively.[26] In a case of RA the common risk calculators for Cardiovascular disease like Framingham under estimate the cardiovascular risk. Therefore, it was advisable to use the risk score models by a multiplication factor of 1.5 for Cardiovascular morbidity and mortality in rheumatoid arthritis patients.[37]

In RA patients, the CVS morbidity and mortality meets two of the following three criteria [38]:

(i) Duration of disease will be >10 years.

(ii)Rheumatoid factor (RF) and or anti-CCP antibodies are positive.

(iii) Presence of extra-articular manifestations.

In RA patients the clinical presentation of cardiovascular disease differ from the general population. Douglas and co-workers were found that more recurrent cardiac events are present in RA patients than in controls.[38]

Increased mortality following cardiovascular events- the "30-days cardiovascular mortality after a first acute cardiovascular event was 17.6% in RA patients vs. 10.8% in non-RA patients".[39]

In RA patients, the overall case fatality is higher compared to controls (hazard ratio 1.67, 95% CI: 1.02–2.71), and also a shorter survival time after a myocardial infarction. These findings are of interest, as similarities are shown to be increased cardiovascular risk in patients with type 2 diabetes.[40]

Inflammation of RA is related to dyslipidaemia. Total cholesterol (TC) and High-density lipoprotein cholesterol (HDL-C) levels are low in RA patients.[41] But when the RA patients treated with anti-inflammatory drugs, the lipid levels become increased,but there is no change in atherogenic index TC/ HDL-C after treatment.[42]

The association between CV risk and Lp (a) in general population was assessed through genetic and Mendelian randomization studies. This strongly point that, Lp(a) as important risk factor for the process of atherogenesis[43].

LIPOPROTEIN(a):

Lipoprotein (a) is a particle,it is associated with increased risk of premature CHD and stroke[20]. Lp(a) is nearly identical in structure to an LDL particle[25]. The distinguishing feature is apo-(a), plasminogen like glycoprotein that is linked toapo B100 by disulphide bond.[44] The structural gene for apo(a) is located on chromosome 6. Lp(a) particles are heterogenous in both size and density, as a result of a differing number of repeating peptide sequences, called kringles,in the apo(a) portion of the molecule. Lp(a)is larger than LDL and has a higher lipid content and a slightly lower density.[20]

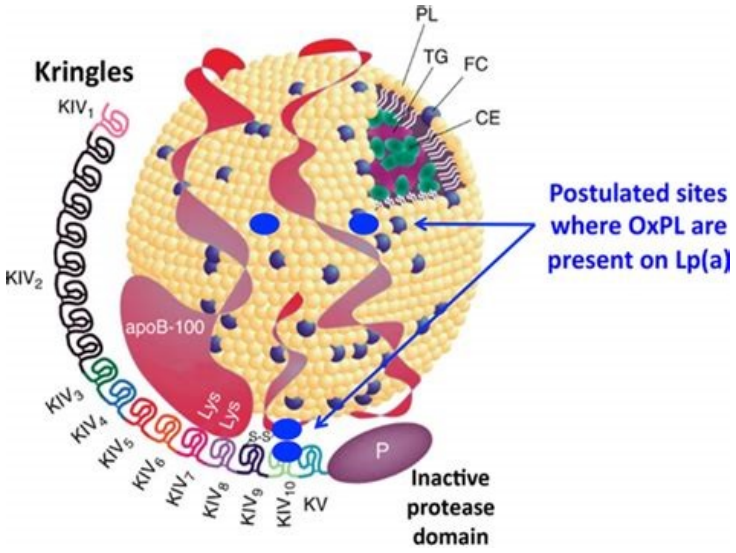


Fig 13:Shows Structure of Lipoprotein (a).

Factors affecting Lp(a):

(1) Genetics- Determine the Circulating levels of Lp(a).

(2) Diet may play some role- trans fatty acids have been shown to increase Lp(a).

(3) Niacin reduces Lp(a) and raises HDL.[25]

Lp(a) was involved in inflammation and thrombosis [4].Lp(a) was significantly associated with acute phase proteins and was found topromote

proliferation of vascular smooth muscle cells and chemotaxis of human monocytes. Its role in atherosclerosis was suggested by the structure of apo (a).[45]This antifibrinolytic effect is primarily defined by the size of the apo(a) polymorphs, which show heterogeneity in their fibrin-binding activity-only small size isoforms display high affinity binding to fibrin.[44]

PATHOGENESIS OF LIPOPROTEIN(A):

In Lp(a), the apo(a) exists several isoforms – defined by variable number of copies of plasminogen- like kringle 4 and single copies of kringle 5 and the catalytic region[46]. At least one of the plasminogen-likekringle 4 copies present in apo(a) (kringle IV type 10) contains a lysine binding site (LBS) that is similar to that of plasminogen. This structure allows binding of these proteins to fibrin and cell membranes[47].

Plasminogen thus bound is cleaved at Arg561-Val562 by plasminogen activators and transformed into plasmin. This mechanism ensures fibrinolysis and pericellular proteolysis[49]. In apo(a) a Ser-Ile substitution at the Arg-Val plasminogen activation cleavage site prevents its transformation into a plasmin-like enzyme[50]. Because of this structural/functional homology and enzymatic difference, Lp(a) may compete with plasminogen for binding to lysine residues and impair fibrinolysis and pericellular proteolysis[48].

Kringle domines of Lp(a) have a high level of homology with plasminogen, (a precursor of plasmin that promotes clot lysis via fibrin cleavage), it has been proposed that Lp(a) may compete with plasminogen for binding sites on endothelium and on fibrin, thereby promoting clotting[20].

The Pathogenesis of LP(A) in development of atherosclerosis are:

(i) It accumulates in the arterial intimal layer.

(ii) It activates inflammatory cells.

(iii) Binds to proinflammatory-oxidized-phospholipids[1].

(iv) It promotes thrombosis and inhibits fibrinolysis due to the high structural homology between apo(a) and plasminogen.[25]

Lipid contents of the Lp (a) and LDL-C were similar. They might have similar mechanisms for development of atherosclerosis. It was suggested that in active RA the reticulo-endothelial system was became over stimulated and the lipid elimination by scavenger receptors of macrophages was increased. For this reason,important atherogenic factor -the LP(a) have vital role[8].

Role of Lipoprotein(a) in atherosclerosis:

Lp(a) is a sticky particle, which binds to glycosaminoglycans, proteoglycans, collagen and other connective proteoglycans, collagen and other connective tissue structures[46].It isimmunochemically related to plasminogen. Plasminogen is a zymogen foundinplasma.Upon activation, itforms plasmin which causes fibrinolysis.Apo(a) moiety [kringle IV &V repeats]ofLp(a)has about 0% amino acid sequence homology to plasminogen[47]. By different ways, Lp(a) interferes with fibrinolytic activity of plasminogen.(i)Because of the structural homology, Lp(a) inhibits the binding of plasminogen to it'sreceptor on endothelial cells. Thus, the activation of plasminogentoplasminis prevented.

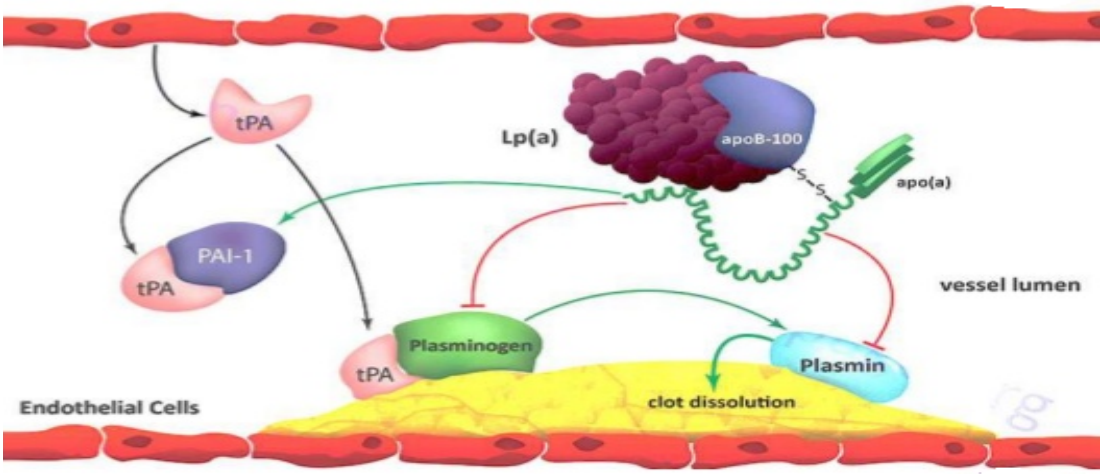


Fig 14: Pathogenesis Lp(a) in atherosclerosis.

(ii) Lp(a)competes with plasminogen for binding sites on fibrin, interferingwiththe action of plasminogenincausingfibrinolysis.

(iii) Lp(a) attaches to lysine residues on fibrin in atherosclerotic plaquesinhibitingfibrinolysis.

Lp(a) levels of about 30mg/dL has traditionally been used as an atherogenic cutoff, elevated levels of Lp(a) (>30mg/dL) are now known to increase the risk ofcardiovascular disease.[11]

Lipoprotein(a)andCardiovasculardiseaserisk:

Lipoprotein(a)isamajorindependentgeneticriskfactorfor cardiovascular disease.[51]ElevatedLp(a)levelsassociatedobustlyandspecificallywithincreasedris k ofcardiovasculardisease.Thisassociationiscontinuousanddoesnotdependon thepresenceofotherCVDriskfactors.Lp(a)levels,likeelevatedLDL,iscausallyrelat

edtoprematuredevelopmentofatherosclerosisandCVD.[52]

The concentration of Lp(a) is inversely related to the size of apo(a) isoform[20]. Studieshaveshown thatsubjectswithsmall apo(a)phenotypeshaveatwofoldriskofCVDandstrokecomparedwiththosewithlargerisoformsofapo(a).[53]

METABOLISMOFLIPOPROTEINS-ANOVERVIEW:

1.LiverandIntestinalmetabolismofTriglyceriderichApoBcontainingLipoproteins:[53]

ApoB-48Chylomicron (CM) particles assembled in the enterocytes (exogenouspathway) and ApoB-100 Very low density lipoprotein (VLDL) particles produced in the hepatocytes (endogenous pathway) are releasedinto thecirculation.These nascent CMandVLDLparticlesacquireApoEand ApoC fromHDL particles in circulation and become functionally mature.ApoCII activatestheenzymeLipoproteinLipase(LPL),while theApoCIandApoCIII haveinhibitory roles.[11,20]LPLhydrolyzes triglycerides in mature CMandVLDL particlesinthe capillaries ofperfused skeletalmuscle andadipose tissue.[53] This leads to therelease of free fatty acids which are taken up by adipocytes/myocytes and CMremnants and VLDL remnants (Intermediate densitylipoprotein[IDL]) areformed. CMremnants are removed by the liver via LDL receptor related protein(LRP), aftertriglyceride andphospholipidsarehydrolyzedbyHepaticLipase(HL)[11].

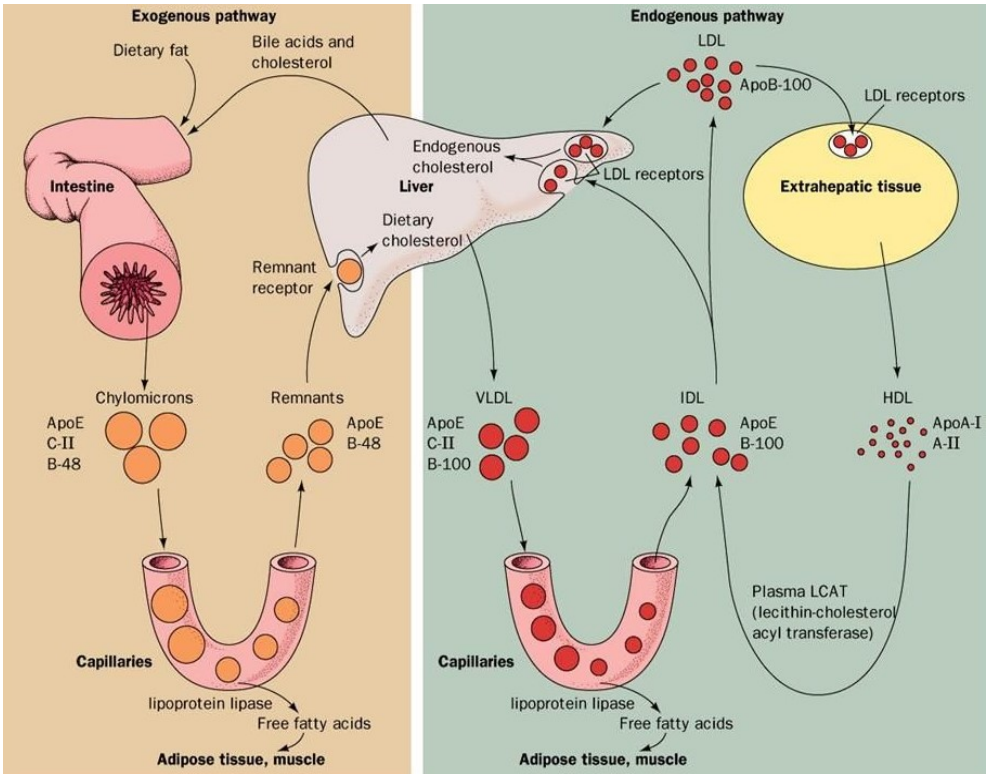


Figure 15: Overview of Lipoprotein metabolism.

MostoftheIDLparticlesaresubjectedtofurtherhydrolysisbyHL tobecomesLowDensityLipoproteins(LDL).LDL particles aretakenupby the LDL receptor. Rest of the IDLparticles are taken up by the LRP. VLDLarealsoremovedviatheVLDLreceptorinadipocytes/myocytes.[11]

2.MetabolismofApoA1andHighDensityLipoprotein:[11,54]

The Reverse Cholesterol Transport process operates to remove theexcesscholesterol from peripheral tissues via the High density lipoproteinstobedisposedintheliver.Thefreecholesterol (FC) effluxes from thecell to thesurface of lipid-poor ApoA1 via the ATP-binding cassette

transporter1 (ABCA1). Nascent ApoA1 particles becomes mature HDLparticlesthroughtheesterification offrecholesteroltocholesteryl-esters(CE)bytheenzymeLecithin-Cholesteryl Acyl Transferase (LCAT).

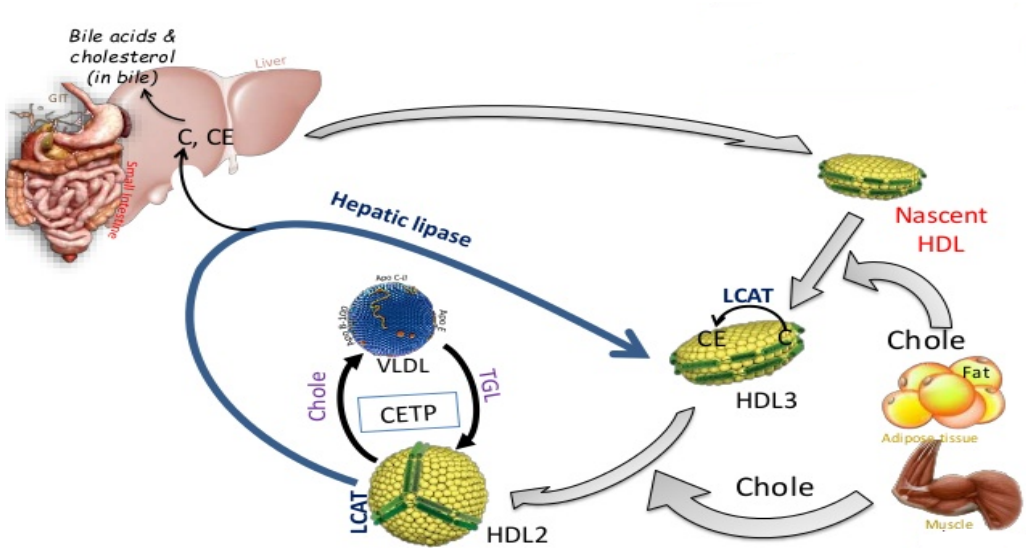


Fig 16: Shows Metabolism of HDL:

CE-richHDLparticlesenterthe circulation[11].Thesearethen, taken updirectly by the liverthrough Hepatic Scavenger Receptor (SR-B1) orindirectlyafter exchange of Cholesteryl Esters inHDL for Triglycerides inApoBcontaining lipoproteins (LDL, VLDL, CM,IDL)byCholesterylEsterTransferProtein(CETP).[55]

Carotid Artery Doppler:

Subclinical atherosclerosis can also be demonstrated by increased carotid artery intima media thickness (IMT)[56]. In patients with high cardiovascular

risk, it is important to identify the vascular injury early. The carotid artery intima-media thickness (IMT), measured by ultrasound, has been established as a valid marker for early atherosclerosis[58].The cIMT measured by ultrasound is a surrogate marker of atherosclerosis and it is the most widely used noninvasive imaging method to assess CVD risk and atherosclerosis[57].

Intima–media thickness (IMT), also called intimal medial thickness. It is a measurement of the thickness of tunica intima and tunica media- the innermost two layers of the wall of an artery. The measurement is usually made by external ultrasound .[59]

Ultrasound IMT measurement was first proposed and validated in vitro by Paolo Pignoli in 1984.[60]. However, in 2003 the European society of Hypertension -guidelines for the management of arterial hypertension recommended the use of IMT measurements in high-risk patients to help identify target organ damage.[61]

Carotid intima media thickness (IMT) is a doppler measurement of carotid artery, that measures the intima media layers- where the atheroma begins. It is considered as a good marker of atherosclerosis in its initial stage.[62]Among the various screening methods the carotid intima media thickness (CIMT) has gained wide acceptance marker of atherosclerosis, predicting future cardiovascular events[63]. CIMT is a easy, non-invasive, reliable and relatively inexpensive tool for screening atherosclerosis.[64]

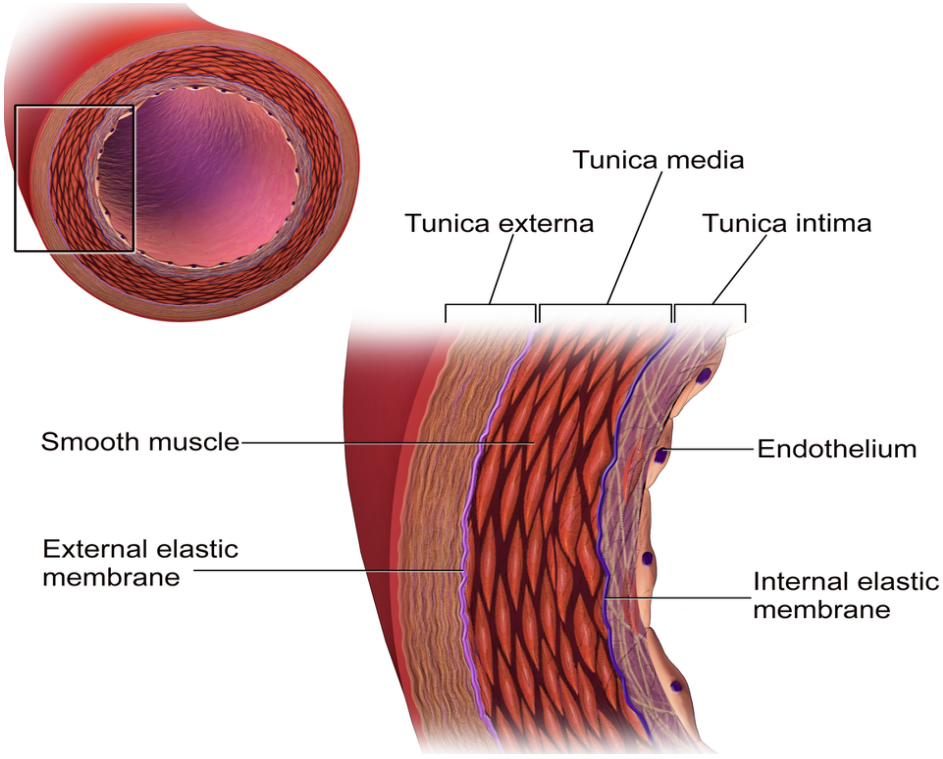


Fig 17 : Show the Structure of an Artery wall

The associations between cIMT and rheumatoid arthritis (RA)

Growing evidence has demonstrated that, patients with RA have a higher risk for atherosclerosis[66,67]. RA patients are associated with enhanced CV risk and subclinical vascular disorder[65]. Recent quantities clinical observations have confirmed that RA is associated with increased CIMT [68].

The mechanisms of RA-related CIM thickening included increased levels of Ox-LDL[69], vWF activity[70], serum mannose-binding lectin[71] as well as

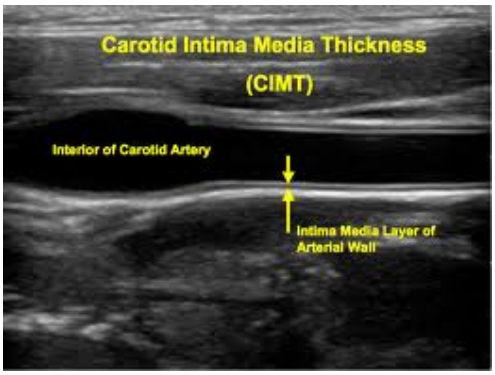


Fig 18: shows normal ultrasonic examination of Carotid IMT

increased levels of inflammation markers such as IL-17 and CRP [72] and lower levels of carotene[70], vitamin D, CD34+ cells[71] and NO[72] and may increase the burden of subclinical atherosclerosis[73]. Their mechanisms involved elevated myeloperoxidase levels in juvenile idiopathic arthritis[74], circulating levels of OxLDL.IMT is used to detect the presence of atherosclerosis in humans and more contentiously to track the regression arrest or progression of atherosclerosis.[75]

Measure of IMT

IMT can be measured by using external ultrasound in large arteries relatively close to the skin (e.g. the carotid, brachial, radial, or femoral arteries). External ultrasound methods have the advantage of being comparatively lowcost and non-invasive and also convenient to the patients.

Deeper internal arteries, such as the coronary arteries require special intravascular catheters employing ultrasound or optical coherence tomography to measure IMT.[76]

External ultrasound methods have the advantage of being comparatively low cost and non-invasive and also convenient to the patients. Deeper internal arteries, such as the coronary arteries require special intravascular catheters employing ultrasound or optical coherence tomography to measure IMT.[76]

The carotid artery is the usual site of measurement of IMT and consensus statements for carotid IMT have been published for adults[77] and children.[78] Often, IMT is measured in three locations: in the common carotid artery (typically at one cm proximal to the flow divider), at the bifurcation, and in the internal carotid artery.[80] IMT measurements of the far (deeper) wall, by ultrasound, are generally considered more reliable than measurements performed on the near (more superficial) wall;[79] although measurement of both near and far wall IMT has also been advocated.[82]

Carotid IMT has been used in many epidemiological and clinical studies and these have shown associations with several risk factors including type-2 DM, familial hypercholesterolemia, High Density Lipoprotein- Cholesterol (HDL-C), triglycerides,[81]**rheumatoid arthritis,**[83]non-alcoholic fatty liver disease.[84]

Since the 1990s, some clinical trials of lifestyle Carotid IMT has been used in many epidemiological and clinical studies and these have shown associations with several risk factors including type-2 DM, familial hypercholesterolemia, High Density Lipoprotein- Cholesterol (HDL-C), triglycerides,[81]**rheumatoid arthritis** and pharmaceutical interventions have also used carotid artery IMT as

a surrogate marker for evaluating the regression and/or progression of atherosclerotic cardiovascular disease.[85]

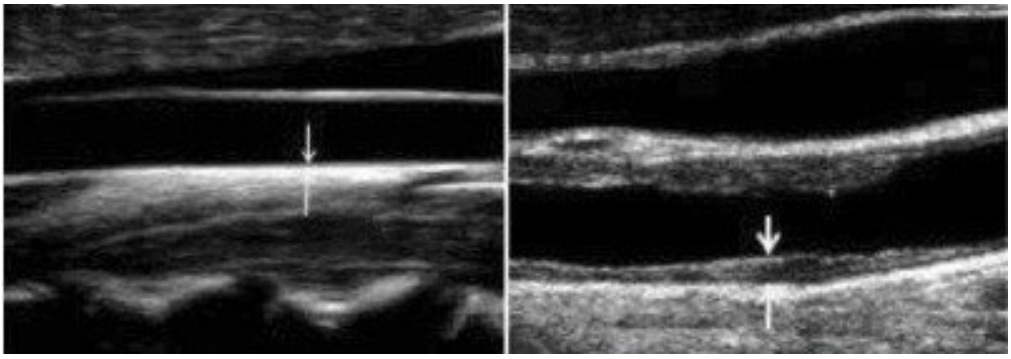


Fig 19(a) Fig 19(b)

Fig 19:show Ultrasonographic examination of Carotid IMT (distance from arrows) at the wall of common carotid artery. (a) Normal Carotid IMT. (b) Increased Carotid IMT.

Although carotid intima-media thickness is strongly associated with atherosclerosis. Intima-medial thickening is a complex process, depending on a variety of factors, including blood pressure, local hemodynamic,shear stress and circumferential tensile stress.[86]

Variations in IMT between different locations (e.g. the common carotid artery, the carotid bulb and the internal carotid artery) may reflect difference in local hemodynamic forces. Ultrasound measurement of carotid intima mediathickness could be recommended to the majority of RA patients, especially in patients classified in the high CV risk group , patients with extra-articular manifestations and RF or anti-CCP antibodies positivity or 10-year disease progression.[87]

The purpose of our present study was to measuring Carotid IMT and Carotid plaques by using high resolution B-mode ultrasound and colour doppler to assess the existence of subclinical atherosclerosis in RA patients and healthy controls.[10]

Hence, we measure fasting serum lipoprotein (a), lipid profile and to measure the carotid artery intima media thickness in RA patients and control groups.

AIM:

To Evaluate the association of Serum Fasting Lipoprotein (a) and lipid profile & Carotid Intima Media Thickness in Rheumatoid Arthritis patients in prediction of Cardiovascular Risk.

OBJECTIVES:

1.To estimate Fasting Lipid Profile & serum Lipoprotein(a) in Controls and Rheumatoid Arthritis patients.

2.To measure the Carotid intima media thickness and Carotid plaques on both Right and left sides.

3.To correlate Serum Lipoprotein(a) levels with Fasting Lipid Profile in RA patients.

4.To correlate Serum Lipoprotein(a) levels with Carotid intima media thickness in Rheumatoid Arthritis patients.

MATERIALS AND METHODS:

STUDY PLACE:

Government Stanley medical college & Hospital, Chennai-01.

1.Department of Biochemistry

2. Department of Rheumatology

3. Department of Radiology

4.Department of Community medicine

Study Design:

Case control study.

Study Population:

Sero-positive rheumatoid arthritis patients of both genders in the age group between 25 and 70 years are considered as case from rheumatology OP.

Age and sex matched without rheumatoid arthritis are considered as controls.

Sample size:

Case -50. Sero positive, 25-70 years of Rheumatoid Arthritis patients.

Control- 50. Age & Sex matched Healthy subjects taken as controls.

Inclusion criteria:

Male and female patients who were diagnosed with rheumatoid arthritis.

Exclusion criteria:

1.Patients with Hypertension, diabetes mellitus and clinically manifest atherosclerosis by way of coronary artery disease, peripheral vascular disease and cerebrovascular disease.

2.Patients known to have dyslipidemia of any aetiology and on treatment.

3. Past History of systemic disease like pulmonary disorder, thyroid disorder, Liver disorder, etc.

4. Age group under 18 years.

5.History of smoking and alcoholism.

Study duration:

December 2017 – July 2018.

METHODS:

Study procedure:

After obtaining institutional ethical committee clearance, the study was started. The purpose of the study and study procedure was explained to the RA patients and control groups with their native language. Informed consent was obtained in both cases and controls. After taking detailed clinical history taking and General &Systemic examination I filled the clinical proforma and the fasting blood samples were collected and the carotid artery doppler was done on the

same day. Fasting lipid profile &Lp(a) levels were estimated and Carotid Intima Media Thickness & Carotid plaques were measured.

Test results were recorded and data was analysed by standard statistical methods.

Sample Collection and Preparation:

After getting informed consent from the patient, 10-12 hours fasting morning blood sample was collected under strict aseptic precautions in a plain red topped venepuncture tubes without any additives or gel barrier. Samples are allowed to clot for 20-30 minutes and centrifuged at 2000-2500 rpm for 15minutes. Serum was separated immediately and aliquoted into 2 Eppendorf's -one aliquot was used for to measure Serum Lipid profile. Other aliquot was stored at -20^0C in deep freezer for estimation of Serum Lipoprotein(a). Lipoprotein (a) levels were stable up to 3months if the serum is stored at -20^0C.

Study population were examined and following estimationswere done in serum samples:

(i) Lipoprotein(a).

(ii) Fasting Lipid profile:

(a)Serum Total Cholesterol(TC)

(b)Serum Triglycerides (TGL)

(c)Serum HDL(HDL)

(d)Serum LDL: calculated using Friedewald equation:

LDL = Total Cholesterol – (HDL + Triglyceride/5)

(e) Serum VLDL = TGL / 5

(f)Non HDL = Total Cholesterol – HDL.

Tests are assayed using kits by fully automated Beckman Coulter AU 480, after Calibration and internal quality control was done.

(iii)CAROTID ARTERY DOPPLER:

Carotid artery intimal and medial thickness measured by color doppler ultrasonography using high frequency linear probe.

METHODS

ESTIMATIONOFSERUMTOTALCHOLESTEROL:[11]

Method:

Enzymatic Colorimetric: Cholesterol oxidase

CHOD–PAP method.

Principle:

Step1:

$$\text{Cholesterolesters} + H_2O \xrightarrow{\text{Cholesterolesterase}} \text{FattyAcids} + \text{Cholesterol}$$

Step2:

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesteroloxidase}} \text{Cholest-4-en-3-one} + H_2O_2$$

Step3:

$$2H_2O_2 + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneiminedye} + 4H_2O$$

The intensity of the pink colour due to quinoneimine dye formed, is DirectlyProportional to Cholesterol concentration.

REAGENT COMPOSITION:

Goods Buffer (pH–6.4):100mmol/L

Cholesterol oxidase:>100U/L

Cholesterol esterase:>200U/L

Peroxidase:>3000U/L

4-Amino antipyrine:0.3mmol/L

Phenol:5mmol/L

SYSTEM PARAMETERS:

Mode	EndPoint
Wavelength1	505nm
Wavelength2	670nm

SampleVolume	10µL
ReagentVolume	1000µL
Incubationtime	5minutes
IncubationTemperature	37°C
NormalLow	50mg/dL
NormalHigh	230mg/dL
Linearity	750mg/dL
Standardconcentration	200mg/dL
Absorbancelimit	0.4
Blankwith	Reagent

ASSAY PROCEDURE:

	Blank	Standard	Sample
Reagent	300µL	300µL	300µL
Distilled water	3µL	-	-
Standard	-	3µL	-
Sample	-	-	3µL

Mix well and incubate for 5minutes at 37°C.Read the absorbance of the Test(T) and standard (S)againstreagentblank at 505nm or Green filter.

Tests were assayed on Beckman Coulter AU 480 autoanalyser afterCalibration.

CALCULATION:

$$\text{Cholesterol concentration(mg/dL)} = \frac{\text{AbsorbanceofTest x Std.con (200mg/dL)}}{\text{Absorbance of Standard}}$$

Referenceintervals:[11,2]

Adults

SerumTotalCholesterol: Desirable:<200mg/dL

BorderlineHigh:200-239mg/dL

High:>239mg/dL

ESTIMATION OF SERUM TRIGLYCERIDES:

Method: Enzymatic Colorimetric:

Glycero-3-phosphateoxidasemethod

GPO-TOPS

Principle:

Step1:

Lipasecatalyzed hydrolysisoftriglyceridestoglycerolandfreefattyacids.

Triglyceride+3H_2OLipaseGlycerol+3Fattyacids

Step2:

Glycerokinasephosphorylatestheglycerolinan ATP-requiringreaction.

Glycerol+ATPGlycerokinaseGlycerol-3-phosphate+ADP

Step3:

Glycerophosphateoxidase(GPO)oxidizesGlycerol-3-phosphateto DihydroxyacetonephosphateandH_2O_2.

Glycerol-3-phosphateGPO Dihydroxyacetonephosphate+H_2O_2+O_2

Step4:

H_2O_2formedismeasuredinaperoxidasecatalyzedreactionthatformsacoloreddye

$2H_2O_2$+4-Aminoantipyrine+TOPS Peroxidase Quinoneiminedye+$4H_2O$

Theintensityofthecolourduetoquinoneiminedyeformed,isdirectly

ProportionaltoTriglycerideconcentration.

REAGENTCOMPOSITION:

PipesBuffer(pH–7.0):5mmol/L

TOPS:5.3mmol/L

PotassiumFerrocyanate:10mmol/L

Magnesiumsalt:17mmol/L

4-Aminoantipyrine:0.9mmol/L

ATP:3.15mmol/L

Lipoproteinlipase:>1800U/L

Glycerol kinase:>450U/L

Glycerol-3-phosphate oxidase:>3500U/L

Peroxidase:>450U/L

TOPS:N-Ethyl-N-sulfopropyl-m-toluidine

SYSTEM PARAMETERS:

Mode of reaction	EndPoint
Slope of reaction	Increasing
Wave length1	546nm(540-560nm)
Wave length2	630nm
Temperature	37°C
Standard concentration	200mg/dL
Linearity	1000mg/dL
Blank with	Reagent
Incubation time	5minutes
ample volume	10µL
Reagent volume	1000µL
Cuvette	1 cm light path

ASSAY PROCEDURE:

	Blank	Standard	Sample
Reagent	300µL	300µL	300µL
Distilled water	3µL	-	-
Standard	-	3µL	-
Sample	-	-	3µL

Mixwellandincubate it for5minutesat 37°C.Readtheabsorbanceofthetestsampleandstandardagainstreagentblank.Tests wereassayedonBeckmanCoulterAU480autoanalyseraftercalibration.

CALCULATION:

Triglyceride concentration (mg/dL)=Absorbance of Test×Stdconc.mg/dl

Absorbance of Standard

Reference interval:[11,20]

Adults :Desirable:<150mg/dL

Male:60-165mg/dLFemale:40-140mg/dL

ESTIMATION OF SERUM HDL CHOLESTEROL:

Method:

DirectHomogenousAssay

Principle:

The Cholesterol from low density lipoproteins (LDL), very low density lipoprotein(VLDL) and chylomicrons (CM)is brokendown by the cholesteroloxidase(CHOD) in an enzymatic accelerated non-color forming reaction. The detergent present in the reagent B, solubilizes cholesterol from High density lipoproteins(HDL) in the sample.The HDL cholesterol is then spectrophotometrically measured by means of the coupled reactions.

Step 1:

Cholesterol esters $+H_2O$ $\xrightarrow{\text{chol. esterase}}$ Cholesterol + Fatty acid

Step 2:

Cholesterol + ½ O_2 $\xrightarrow{\text{chol.oxidase}}$ Cholesternone $+H_2O_2$

Step3:

$2H_2O_2$+4-Aminoantipyrine+TODB $\xrightarrow{\text{Peroxidase}}$ Quinoneimine + $4H_2O$

(λmax=560nm)

REAGENTCOMPOSITION:

R1Reagent:Good's Buffer, cholesterol esterase, cholesterol oxidase, peroxidas,N,N-bis(4-sulfobutyl)-m-toluidine.

ASSAYPROCEDURE:

4µLofsample+300µLofReagent1

Incubatefor5minutesat37°C

Measureabsorbance1bichromaticallyat660/546nm

Add100µLofReagent2

Incubatefor5minutesat37°C

MeasureAbsorbance2bichromaticallyat660/546nm

Calculate HDL concentration by using

ΔAbsorbance = Absorbance 1 -Absorbance 2

OtherAssayParameters:

Standardconcentration:49mg/dL

Linearity:200mg/dL

Blankwith:Reagent

Reagentblankabsorbance:<0.05

Cuvette:1cmlightpath

TestswereassayedonBeckmanCoulterAU480autoanalyserafterCalibration.

CALCULATION:

$$\text{HDL concentration (mg/dL)} = \frac{\Delta\text{Absorbance of sample} \times \text{Std conc. mg/dl}}{\Delta\text{Absorbance of standard}}$$

Reference intervals:[11,20]

Adults

Serum HDL: 40-60 mg/dL

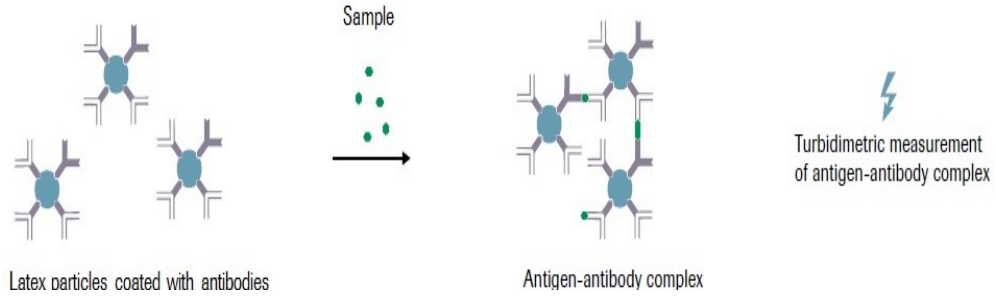
DETERMINATION OF SERUM LIPOPROTEIN(a):

Method:

Immunoturbidimetry

Principle:

Latex particles coated with anti Lp(a) antibodies agglutinate with Lp(a) in samples. This agglutination causes a change in absorbance, depending on the Lp(a) content in the sample, which is quantified by comparing with a calibrator of known Lp(a) concentration.



REAGENTS:

ReagentR1:

Glycinebuffer(pH-8.3):50mmol/L

ReagentR2 :

Glycinebuffer(pH-8.2)

Latexparticlescoatedwithanti-humanLipoprotein(a) antibody (rabbit)

Sodiumazide:0.9g/L- as preservative.

System parameters:

Mode	Multi-PointCal.
Reactiontype	Ascending
Wavelength	700nm
Blankwith	Distilledwater
Samplevolume	7µL
Reagentvolume	500µL
Delaytime	10seconds
Readtime	240seconds
Linearity	90mg/dL

CALIBRATOR:

TheCalibratorisalyophilizedserumofhumanorigincontainingLp(a)ofknowncon centrationof96.6mg/dL,afterreconstitutionwith1mLNaCl9g/L.ThefollowingLp (a)calibratordilutionsinNaCl9g/Lwereprepared.The

concentrationofeachoftheLp(a)calibratorswasobtainedbymultiplyingtheLp(a)concentration–96.6mg/dLbythecorrespondingfactor.

Calibratordilution	1	2	3	4	5
Lp(a)Calibrator(µL)	-	25	50	75	100
NaCl9g/L(µL)	100	75	50	25	-
Factor	0	0.25	0.5	0.75	1.0
ConcentrationofLp(a) Calibratordilution(mg/dL)	0	24.15	48.30	72.45	96.60

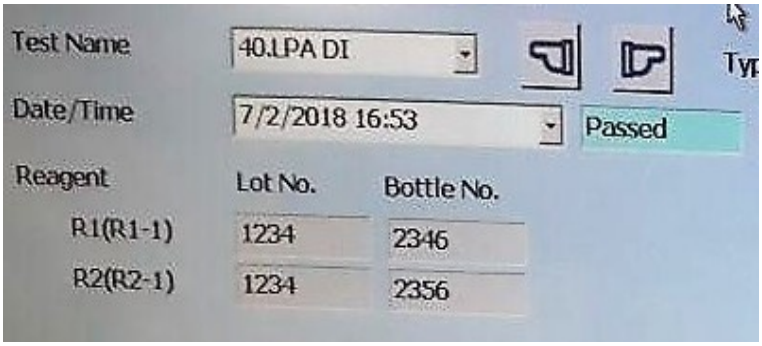
ASSAYPROCEDURE:

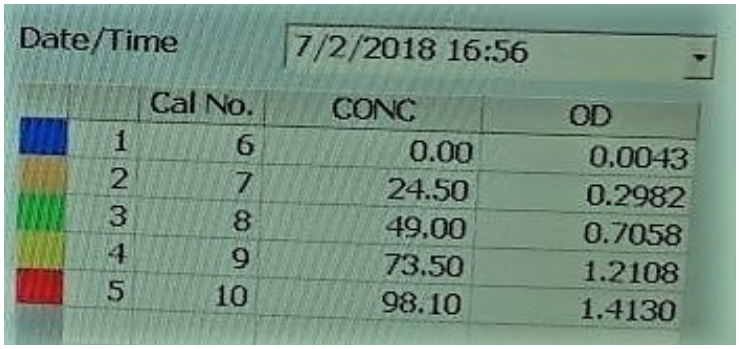
Thereagentsarebroughttotemperatureof37°C.

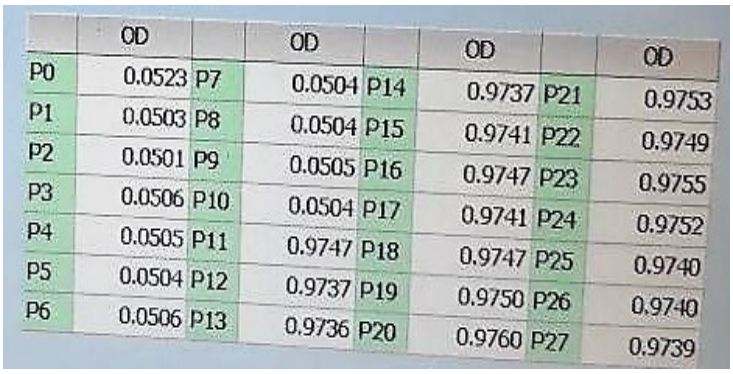
	Blank	Calibrator	Sample
Distilledwater	7.5µL	-	-
ReagentR1	300 µL	300µL	300µL
ReagentR2	150µL	150µL	150µL
Calibrator		7.5µL	-
Sample		-	7.5µL

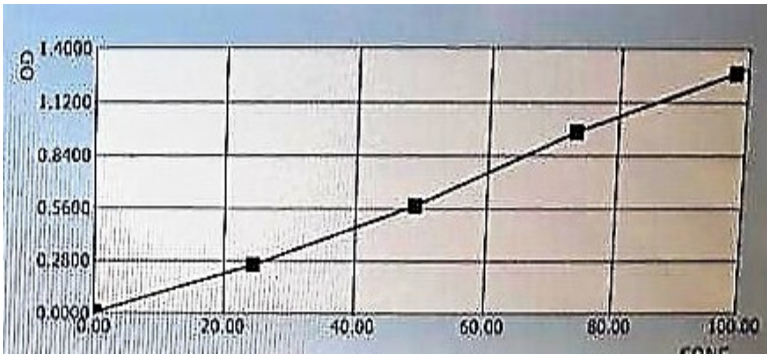
Mixandreadtheabsorbance(A_1)with in 30sec, incubate for 5minutes then read absorbance(A_2)

CALIBRATIONREPORT:









Quality control:

Quality control pool values are within the established ranges.

RADOX InternalQualityControlforImmunologicalassays

Mean:32.5Standarddeviation:1.38

CoefficientofVariation:10.35%Range:30to49.09mg/dL

CALCULATIONS:

The absorbance differences (A2 – A1) of each Lp(a) calibrator Was calculated and the values obtained against the corresponding Lp(a) concentrations were plotted in a calibration curve.

Lp(a)concentrationinthesamplewascalculatedbyinterpolationofit's(A2-A1)inthecalibrationcurve.

Reference interval:[11,20]

SerumLp(a):

Desirablecut-off:<30mg/dL.

CALCULATIONS:

Theabsorbancedifferences(A2-A1)ofeachLp(a)calibratorwascalculatedandthevaluesobtainedagainstthecorrespondingLp(a)concentrationwere plottedinacalibrationcurve.Lp(a)concentrationinthesamplewascalculatedbyinterpolationofit's(A2-A1)in the calibration curve.

Reference interval:[11,20]

SerumLp(a):

Desirablecut-off:<30mg/dL.

CAROTID ARTERY DOPPLER:

Patient was placed on supine position, with the head turned away from the sonographer. The common carotid artery on both sides were carefully examined for wall changes in all subjects, obtaining different longitudinal and transverse views with the high-resolution B-mode ultrasound equipment Samsung Accuvix X-G equipped by liner probe (7.5 10 Hz). A region about 1.5cm proximal to the carotid bifurcation was identified, and the intima media thickness (IMT) of the far wall was evaluated as the distance between the luminal-intimal interface and the medial adventitial interface.

All ultrasound measurements were performed by the same examiner who was unaware of subject characteristics. This evaluation aims to determine the intima media thickness (IMT) and to detect carotid plaques. The mean IMT (the mean of both right and left sides) was assessed. At the same time the maximum IMT (the highest value either right or left) was also assessed.

IMT is considered abnormal if >0.07cm.[88]

Plaques were defined as focal widening relative to adjacent segments, with protrusion in to the lumen of calcified or noncalcified material.

RESULTS AND STATISTICAL ANALYSIS

This study was done to evaluate fasting Serum lipoprotein(a), lipid profile levels and right &left side carotid IMT measurement was done in total of 100 subjects, of which 50 with known RA were taken as cases and 50 individuals without RA were taken as controls.

STATISTICAL ANALYSIS

Results of clinical and biochemical profile obtained in patients with Rheumatoid Arthritis disease were compared with those of the control group by statistical analysis using Excel software. Student's unpaired 't' test was used to compare the means between two independent groups. F test was applied between the study variables to know whether 't' test can be applied to study the parameters and also which type of 't' test -either equal variance or separate variance unpaired 't' test can be applied in this study.

Pearson coefficient of correlation was used to estimate the degree of association between two quantitative variables. A p-value of <0.05 will be considered as statistically significant.

Table 1:Age distribution between Study Population.

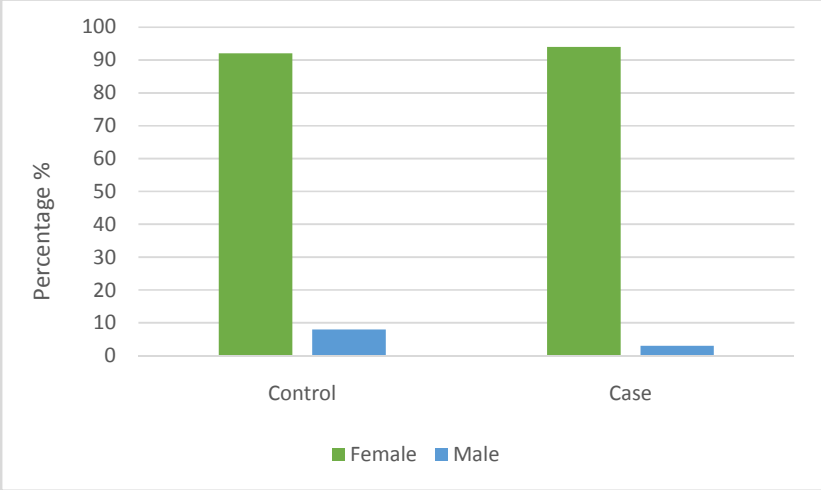
Parameter	Mean ± SD		'P' Value
	Controls (n=50)	Cases (n=50)	
Age	44.7 ± 8.4	47.0 ± 9.1	0.19

Table 1: Shows Age distribution into study group and control group. It shows no significant difference in age between two groups. This study groups are comparable.

Table 2: Gender distribution among Control & RA subjects.

Gender	Control	Case
Females	46 (92%)	47 (94%)
Males	4 (8%)	3 (6%)
Total	50 (100%)	50 (100%)

Graph 1: Gender distribution among Control group & RA Subjects.

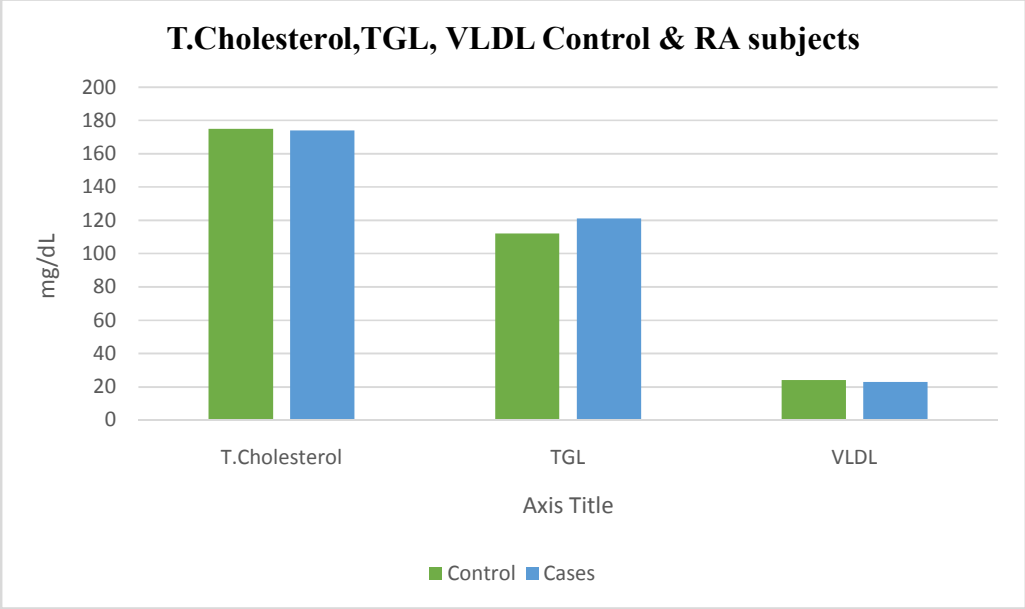


Graph 1& Table 2: Shows the gender distribution among RA subjects and controls. In our study, 94% of RA were females & 6% were malesand in healthy controls 92% of them were females & 8% were males.

Table 3: Comparison of Lipid profile Parameters between Controls and RA subjects.

Fasting Lipid Profile Parameters	Mean ± SD		'P' Value
	Controls (n=50)	Cases (n=50)	
Total Cholesterol (mg / dL)	175±32.1	174 ±27.8	0.90
Triglycerides (mg/dL)	112 ±31.0	121 ± 33.4	0.21
VLDL (mg/dL)	24± 8.5	23 ±6.7	0.93
LDL (mg/dL)	106±23.6	111±30.8	0.40
HDL (mg/dL)	46±11.1	45±9.2	0.69
Non HDL (mg/dL)	131±26	130±33	0.88

Graph 2: Comparison of Total Cholesterol, Triglycerides, VLDL values between Controls and RA subjects.



Graph 3: Comparison of LDL, HDL &NonHDL values between controls and RA subjects.

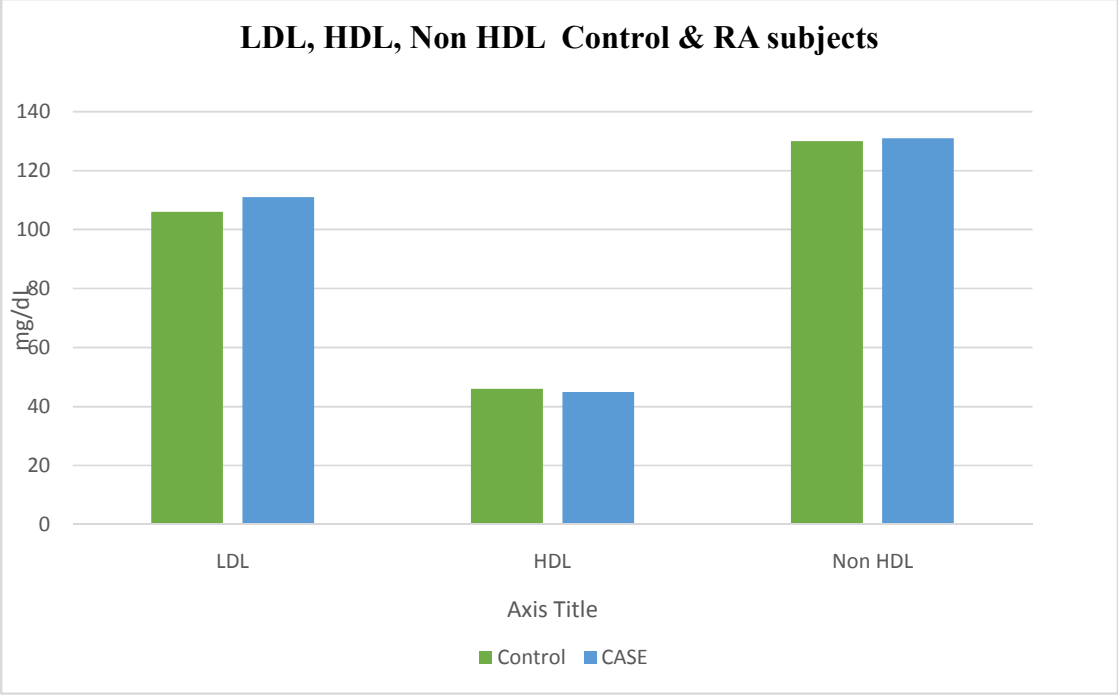


Table 3 & Graph 2,3: Shows that the comparison of lipid profile variables between controls and RA subjects. These data shows there is no statistically significant difference in Total Cholesterol, Triglycerides, VLDL, LDL, HDL, Non HDL values between controls and RA subjects.

Table 4: Comparison of Lipoprotein (a) levels between control and RA subjects.

Parameter	Mean ± SD		'P' Value
	Controls (n=50)	Cases (n=50)	
Lp(a) (mg / dL)	17.3±11.0	32.6 ±17	<0.0001***

***Statistically significant**

<u>Graph 4:</u> Comparison of Lp(a) value between control and RA subjects.

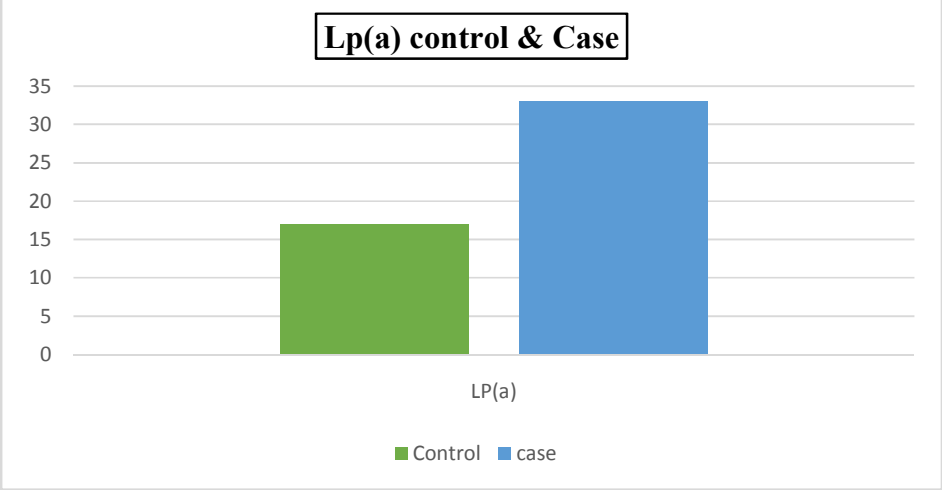


Table 4 & Graph 4: Shows that, the comparison of Lipoprotein(a) between Controls and RA subjects. These data show statistically significant ($p<0.0001$) difference between RA patients and control groups in Lp(a) values between controls and RA subjects.

Table 5: Comparison of Carotid IMT Right and Left side of control and RA subjects.

Measurement	Mean ± SD		'P' Value
	Controls (n=50)	Cases (n=50)	
IMT Right (mm)	0.36±0.1	0.54± 0.14	<0.0001***
IMT Left (mm)	0.39 ± 0.1	0.52 ± 0.13	<0.0001***

***Statistically significant.**

Graph 5: Comparison of Carotid IMT Right and Left side of Control and RA subjects.

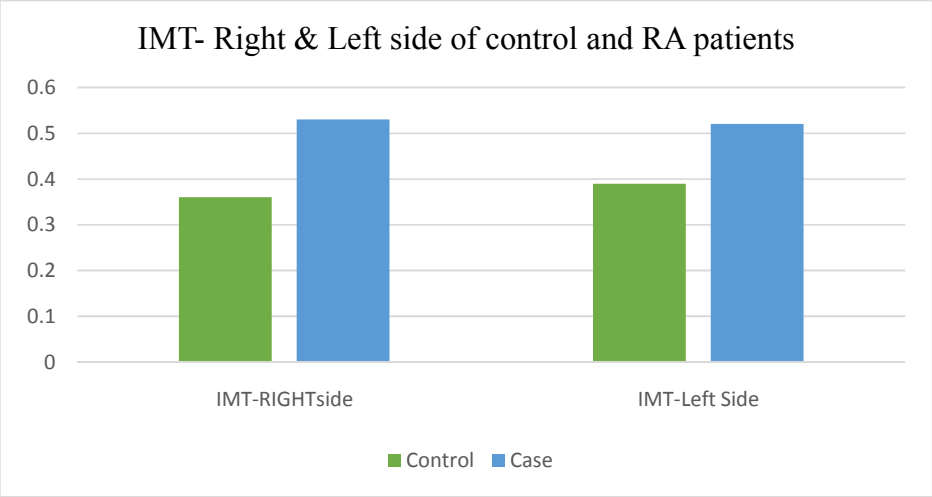


Table 5 & Graph 5: Show the comparison of Carotid IMT of Right & Left sides between controls and RA subjects. The data demonstrate that statisticallysignificant ($p<0.0001$)differences in the values of Carotid IMT of both sides between controls and RA subjects.

Table 6: Pearson's correlation between Serum Lp(a) and Lipid profile in RA patients.

S.No	Analytes	Pearson's correlation coefficient ('r' value)	Significance
1.	Lp(a) Vs TC	0.212	Weak correlation
2.	Lp(a) Vs TGL	0.021	No correlation
3.	Lp(a) Vs LDL	0.361	Moderate correlation
4.	Lp(a) Vs HDL	0.066	No correlation
5.	Lp(a) Vs VLDL	0.015	No correlation

Table 6. Explain the Pearson's Correlation Coefficient between Lp(a) and lipid profile in RA patients. It revealed moderate positive correlation between Serum Lp(a) andLDL($r=0.361$). A weaker positive correlation is observed between serum Lp(a) and Total

cholesterol(r=0.212).Nocorrelation between serum Lp(a) and TGL (r=0.021), VLDL(0.015)and HDL (0.066).

r value = >0.7 Strong correlation.

r value = 0.3 to 0.7 Moderate correlation.

r value = < 0.3 Weak correlation.

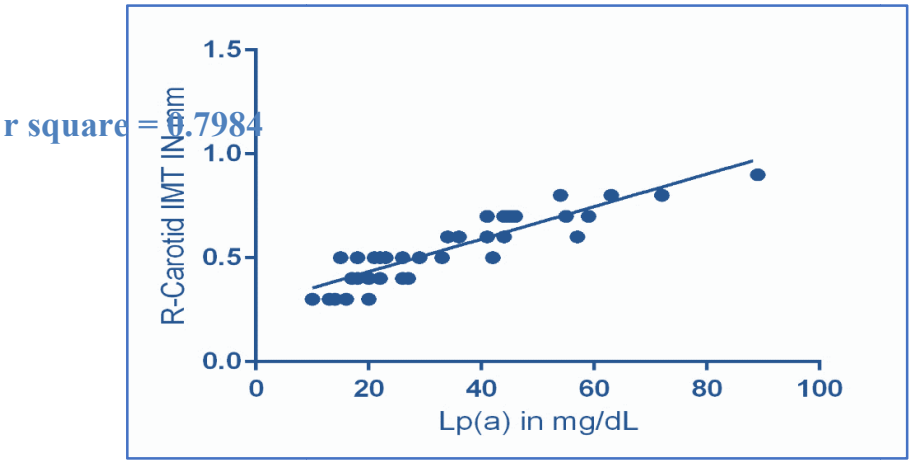
r value = 0 – <0.1 No correlation.

Table 7: Pearson's correlation between Serum Lp(a) and Carotid IMT in RA patients.

S.No	Analytes	Pearson's correlation coefficient ('r' value)	Significance
1.	Lp(a)Vs IMT-Right	0.897	Positive correlation
2.	Lp(a) Vs IMT-Left	0.805	Positive correlation

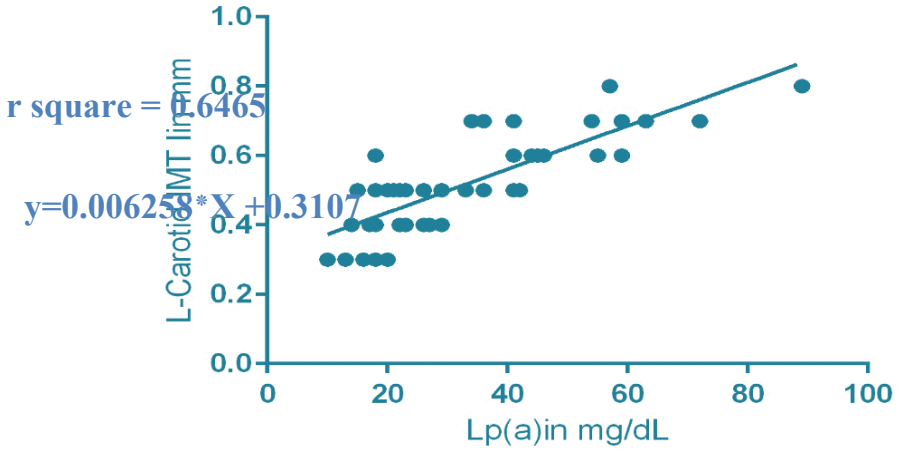
Table 7: Explain the Pearson's Correlation CoefficientbetweenLp(a) and Carotid IMT of right and left sides in RA patients. It demonstrates that a strong positive correlation between Serum Lp(a) and IMT-Right (r=0.897) &IMT-Left (r=0.805) sides of carotid artery.

<u>Graph 6:</u> Regression analysis between Lp(a) levels and Carotid IMT on right side in RA subjects.



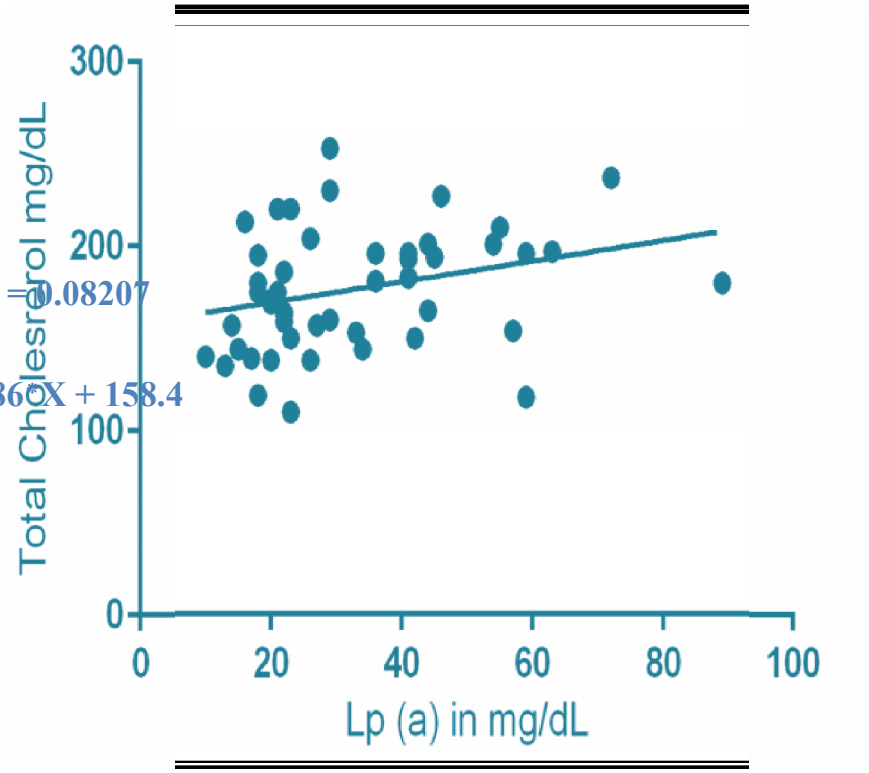
Graph 6 explains the correlation of Serum Lp(a) levels and Carotid IMT on right side among RA subjects. Linear regression analysis has an upward slope suggesting that Right side Carotid IMT values have strong positive correlation (r=0.897) with Serum Lp(a) levels.

<u>Graph 7:</u> Regression analysis between Lp(a) levels and Carotid IMT on left side in RA subjects.



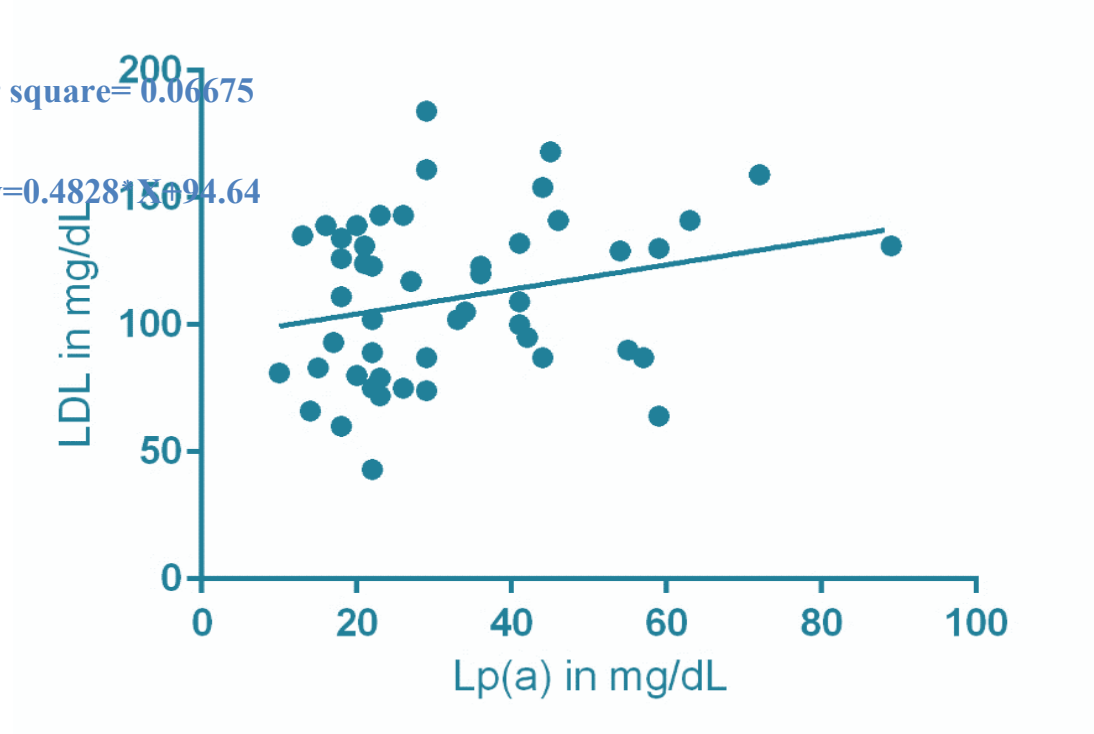
Graph 7 explains the linear regression analysis between Serum Lp(a) levels and Carotid IMT on left side, this demonstrate that the Carotid IMT-Left have a strong positive correlation(r=0.805) with serum Lp(a) levels and linear regression analysis has an upward slope.

<u>Graph 8:</u> Regression analysis between Serum Lp(a) and Total Cholesterol levels in RA subjects.



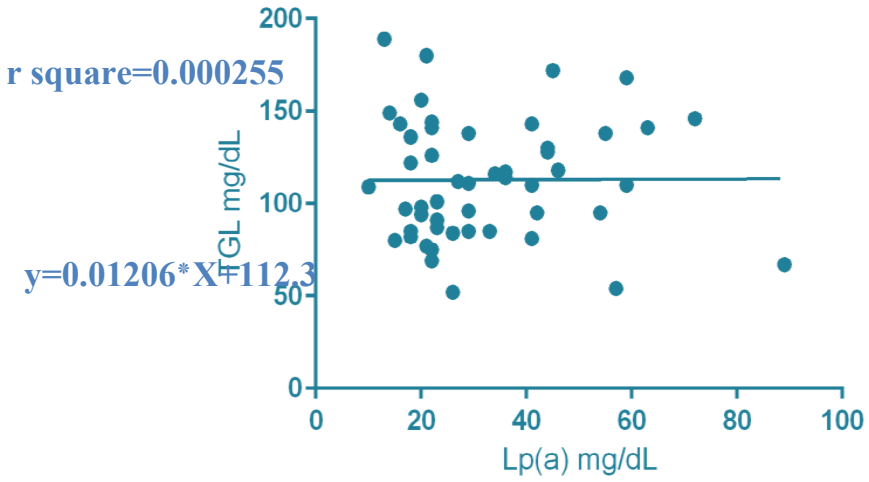
Graph 8 explains the linear regression analysis between Serum Lp(a) and Total cholesterol levels, demonstrate that the Serum Total cholesterol levels have a weak positive correlation (r=0.212) with serum Lp(a) levels.

<u>Graph 9:</u> Regression analysis between Serum Lp(a) and LDL levels in RA subjects.



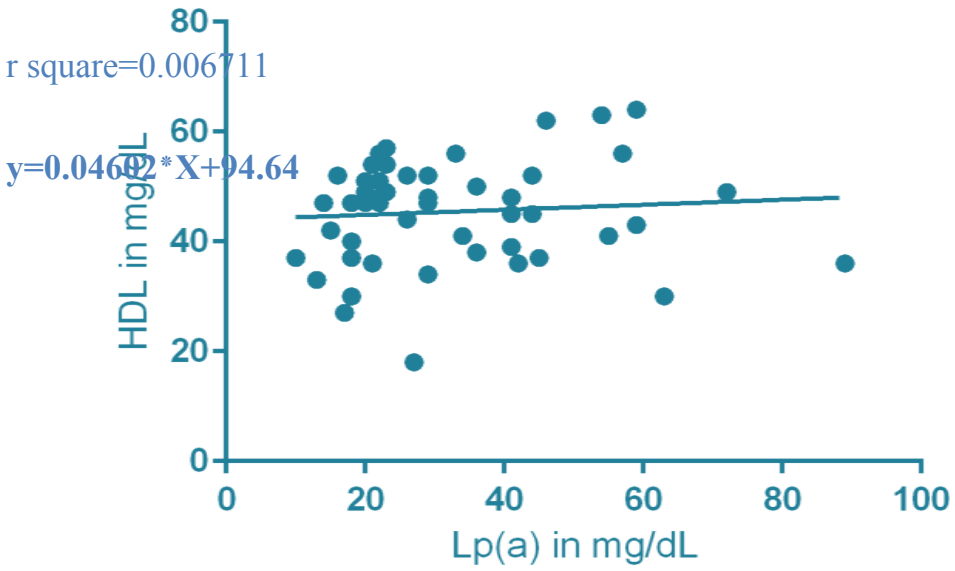
Graph 9 explains the linear regression analysis between Serum Lp(a) and LDL level, demonstrate that the Serum LDL levels have a moderate positive correlation (r=0.361) with serum Lp(a) levels and linear regression analysis has an upward slope.

<u>Graph 10:</u> Regression analysis between Serum Lp(a) and TGL levels in RA subjects.



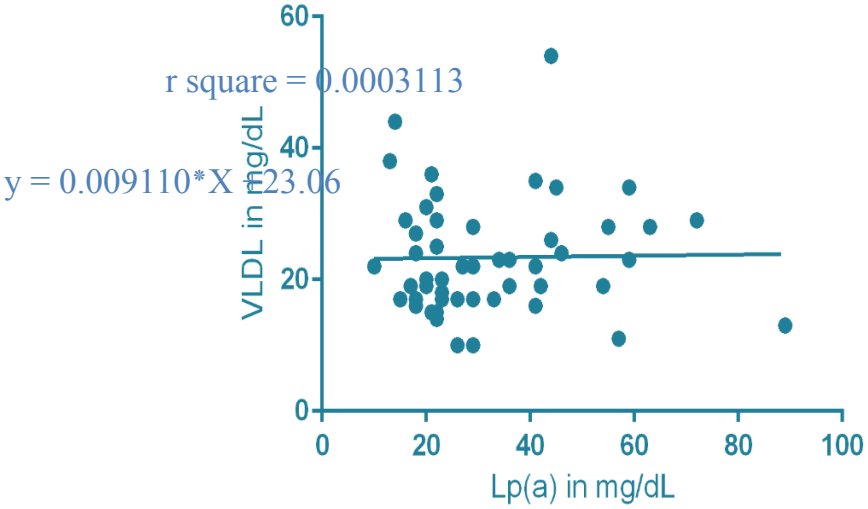
Graph 10 explains the linear regression analysis between Serum Lp(a) and TGL levels, this demonstrates that the Serum TGL has a no correlation(r=0.021) with serum Lp(a) levels.

<u>Graph 11:</u> Regression analysis between Serum Lp(a) and HDL levels in RA subjects.



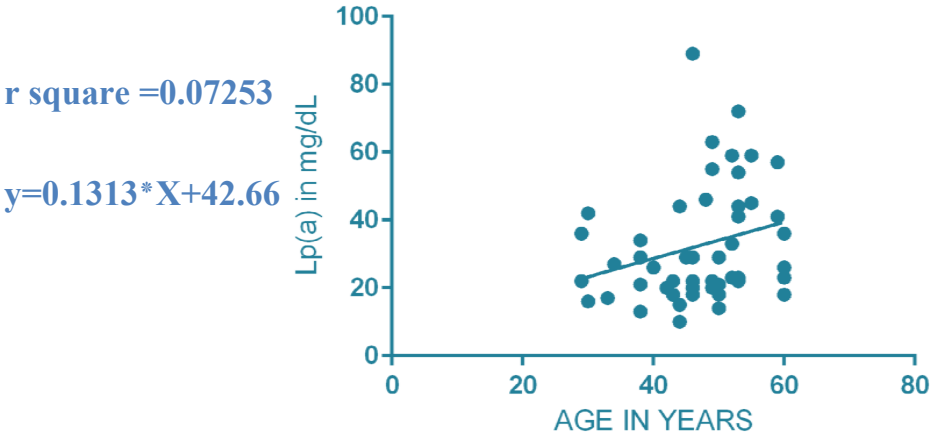
Graph 11 explains the linear regression analysis between Serum Lp(a) and HDL levels, demonstrate that the serum HDL levels have a no correlation (r=0.066) with serum Lp(a) levels.

<u>Graph 12:</u> Regression analysis between Serum Lp(a) and VLDL levels in RA subjects.



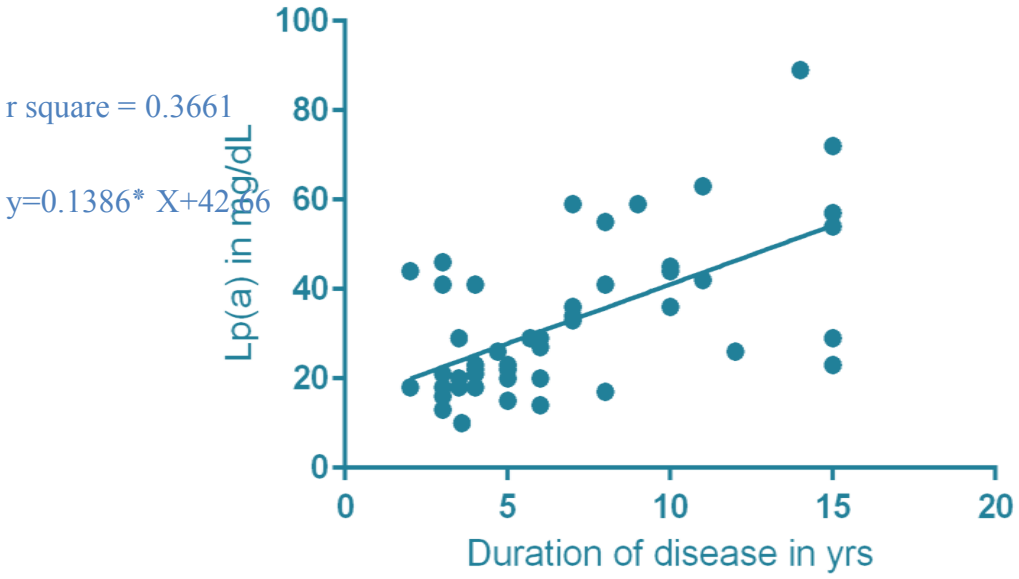
Graph 12 explains the linear regression analysis between Serum Lp(a) and VLDL levels, this demonstrate that the Serum VLDL have a no correlation(r=0.015) with serum Lp(a) levels.

<u>Graph 13:</u> Regression analysis between Serum Lp(a) and RA patients Age.



<u>Graph 13</u>: Explains correlation of serum Lp(a) values and age of the patients. Serum Lp(a) values have positive correlation(r=0.2526) with Age of the patients.

<u>Graph 14:</u> Regression analysis between Duration of disease RA and Lipoprotein (a).



Graph 14: Explains correlation of Lp(a) with Duration of RA. Lp(a) values have moderate positive correlation(r=0.5994) with duration of rheumatoid arthritis.

Table 8: Pearson's correlation between Age of RA patients and carotid IMT on right and left sides.

S.No	Analytes	Pearson's correlation coefficient ('r' value)	Significance
1.	Age Vs IMT-Right	0.3143	Positive correlation
2.	Age Vs IMT-Left	0.219	Positive correlation

Table 8: Explain the Pearson's Correlation Coefficientbetween Age and Carotid IMT of right and left sides in RA patients. It demonstrates that a weak positive correlation between age and IMT-Right (r=0.314) &IMT-Left (r=0.219) sides of carotid artery.

Table 9: Pearson's correlation between Duration of RA and carotid IMT on right and left sides.

S.No	Analytes	Pearson's correlation coefficient ('r' value)	Significance
1.	Duration Vs IMT-Right	0.4599	Positive correlation
2.	Duration Vs IMT-Left	0.4734	Positivecorrelation

Table 9: Explain the Pearson's Correlation Coefficientbetween Duration and Carotid IMT of right and left sides in RA patients. It demonstrates that a moderate positive correlation between duration of disease and IMT-Right (r=0.4599) &IMT-Left (r=0.4734) sides of carotid artery.

DISCUSSION

RA patients are more prone to develop atherosclerosis. There is an necessity for highly sensitive biomarkers for the early detection of CVD in RA since there are fewer diagnostic tools are available to identify the cardiovascular risk in RA patients.

The present study evaluates the cardiovascular risk in RA patients by estimation of Lp(a) level and finds its association with carotid Intima Media Thickness and also to evaluate the association between serum Lipoprotein (a) and lipid profile among subjects with RA and compare the findings with those of healthy control subjects. Adhering strictly to the inclusion and exclusion criteria, 50 cases of Rheumatoid Arthritis and 50 apparently healthy control subjects participated in this study.

The mean age distribution in RA population was 47.0 ± 9.1years which was more or less similar to the mean age distribution in controls was 44.7± 8.4 years. There was no statistically significant difference in age among cases and controls and hence both the groups are comparable.

The sex distribution in the study groups included predominant female subjects which proves that RA is common in women[89]. In RA group, 47 were females & 3 were males and in control group there were 46 females & 4 males. There was no observed difference in sex distribution between RA patients and controls group.

Controls did not meet the classification criteria for RA or any other inflammatory disease. Control subjects were frequently matched for age and sex with the entire group of RA patients so as to ensure that the control group would not differ markedly from RA groups.

In our study, we did not get a significant difference in serum Total Cholesterol [p value:0.90], Serum TGL [p:0.21], LDL [p value:0.85] and VLDL [p value:0.93]. This is in concordance with studies done by Govindan et al[4] and RawhyaR.Elsheroof et al[6]. This might be due to use of DMARDs which lower the cholesterol synthesis[90]. Another reason for getting this result was low number of sample size.

In this study, we also observed no statistically significant difference in HDL [p levels :0.69], between cases and controls. This is in accordance with findings of RawhyaR.Elsheroof et al[6] and Angelile et al[8].

In our study, a statistically significant difference was observed in serum Lipoprotein (a) levels (p value <0.05) between RA patients (32.6 ± 17 mg/dL) and controls (17.3 ± 11.0 mg/dL). This result is concordance with Rantapaa-Dahlquvis et all and Dursunoğlu D[15] et all who also found the similar results in his study. In RA patient serum Lp(a) levels ranges from 10mg/dL to 89mg/dL & in controls ranges from 4.7 mg/dL to 72 mg/dL. The effect of the inflammatory process on Lp(a) metabolism are unclear.

It was suggested that increased synthesis and /or decreased destruction of Lp(a) or changes in Lp(a) distribution between intravascular and extravascular regions may cause dyslipoproteinemia in RA[92].

As an increased concentration of Lp(a) was an important cause of cardiovascular disease in patients with rheumatoid arthritis. Lp(a) is an independent risk factor for CVD in RA patients because Lipoprotein (a) shares structural homology with plasminogen and plasmin. It has potentially prothrombotic and anti-fibrinolytic properties. This could promote clot stabilization and thrombosis.[93]

The present study revealed increase level of carotid IMT in RA patients (Right side is 0.54± 0.14mm & Left side is 0.52 ±0.13mm) when compared to controls (Right side is 0.36 ± 0.1mm & Left side is 0.39±0.1mm) which is statistically significant (p value<0.05). This finding is consistent withmCarotti and salaffi et al[10] studies. Current study results revealed that right sided carotid artery is thicker than left side in RA patients. In control groups left side carotid artery is thicker than right side. Carotid Plaques were also more frequently observed in RA patients. In our study, the cut-off point of IMT is considered abnormal if >0.07cm which was in agreementwith Elshereef RR, Darwish et al[6].

The current study revealed a strong positive correlation between Serum Lipoprotein(a) and Carotid Intima Media Thickness (r=0.897 on right side & r=0.805 on left side) in RA subjects.It indicates that serum Lipoprotein (a) levels are increased as the values of carotid intima media thickness are increased. This result is in concordance with Arpita basu et al who also found same significant correlation between Lipoprotein(a) and carotid IMT in Type-1 DM population.[2]

The present study shows a moderate Positive correlation between Serum Lipoprotein(a) and LDL (r=0.361) in RA subjectsThe present study revealed Weak Positive correlation between Serum Lipoprotein(a) and Total Cholesterol(r=0.212)in RA subjects.

In this study no correlation was found between serum Lp(a) and TGL(r=0.021), VLDL(r=0.015) and HDL (r=0.066) levels in RA subjects. This finding is in accordance with Shiva Govindan et al[4].

This study also revealed the weak positive correlation between age of RA patient and serum Lp(a) levels (r= 0.2526). Lp(a) levels are not dependent on age of the patient.

Thepresent study revealed a moderate Positive correlation between Serum Lipoprotein(a) and duration of RA disease (r=0.5994). This finding is observed with Park Y B et al[66] and Dursunoğlu D[15]studies,indicates that duration of disease increased, the serum Lp(a) levels also increased.

This study revealed positive correlation between duration of disease and carotid IMT. When duration of disease increased carotid IMT also increased.This finding is observed with Park Y B et al[66] studies.

Estimation of serum Lipoprotein(a) is better than lipid profile in early detection of CV risk in RA patients. Increased serum Lp(a) value show good correlation with Carotid Intima Media Thickness in RA patients.

Duration of disease has good correlation with increased level of lipoprotein(a) and carotid intima media thickness. Disease duration is the best predictor of atherosclerosis development in rheumatoid arthritis patients. There were no prior studies that linked Serum Lipoprotein (a) levels and Carotid Intima Media Thickness in Rheumatoid Arthritis patients to evaluate the cardiovascular risk.

CONCLUSION

Inflammatory process of RA simultaneously initiates cardiovascular damage and hence early diagnosis of RA is important along with the recognition of increased risk of CVD. We have shown an elevated level of Lp(a) in RA patients. As the duration of disease increased, Lp(a) levels also increased. Lp(a) should be integrated and monitored as independent CV risk factor in existing screening tests and treatment algorithms of RA patients.

Carotid intima media thickness yields a high predictive power for the development of CV events in RA patients. Carotid ultrasonography could be simple non-invasive method of identifying preclinical atherosclerosis.

In our study no statistically, significant difference in fasting lipid profile in RA patients but serum Lp(a) levels are increased. Hence absence of abnormal lipid profile does not rule out the possibility of coronary vascular disease in these patients.

The results from the present study support, the use of Carotid ultrasonography as a predictor of CV events in RA patients. Subclinical atherosclerosis [increase CIMT, Lipoprotein(a)] is common in RA and correlated well with disease duration. So, every patient of RA should be evaluated for atherosclerosis.

SUMMARY

The study of association between Serum Lp(a) and Carotid intima Media Thickness and Fasting lipid profile among RA subjects who attended the regular OPD was conducted in our institution.

Adhering strictly to the inclusion and exclusion criteria, 50 cases were selected for study. Among them, 47 were females and 3 were males. RA more common in females. 50 healthy control subjects were selected for study with equivalent age distribution for comparison of study parameters.

No interference was done in their routine treatment during the study.

Serum Fasting lipid profile were estimated after standardization using IDMS reference calibrator and internal quality control was done. Serum Lipoprotein (a) levels were estimated by Immunoturbidimetry method. Measuring Carotid Intima Media Thickness on both side of common carotid artery – average was taken. Presence of Carotid plaque was also measured.The Correlation between Fasting Serum Lp(a), lipid profile and IMT was studied.

To conclude, fasting serum Lipoprotein(a) is positively correlated with Carotid Intima media thickness in Rheumatoid Arthritis patients. Hence Serum Lipoprotein (a) and Carotid Intima Media Thickness can be used for the early detection of atherosclerosis and Cardiovascular risk in RA patients.

LIMITATIONS OF THE STUDY

Further study should be taken to overcome the following limitations.

1.Inclusion of the samples from various geographical distribution.

2.Large sample size will give more information on lipid profile.

3. A long term follow up study can provide much more information about the complications of atherosclerosis.

SCOPE OF THE STUDY.

The findings of this study are only suggestive. A longitudinal study with large sample size may be helpful to arrive the spectrum of atherosclerosis changes and to evaluate the mortality and morbidity in RA patients.

ANNEXURE

BIBILIOGRAPHY

1.Missala I, Kassner U, Steinhagen-Thiessen E. A systematic literature review of the association of lipoprotein (a) and autoimmune diseases and atherosclerosis. International journal of rheumatology. 2012;2012.

2.Basu A, Jenkins AJ, Stoner JA, Zhang Y, Klein RL, Lopes-Virella MF, Garvey WT, Schade DS, Wood J, Alaupovic P, Lyons TJ. Apolipoprotein-defined lipoprotein subclasses, serum apolipoproteins, and carotid intima-media thickness in T1D. Journal of lipid research. 2018 May 1;59(5):872-83.

3.Loreto Carmona, Marita Cross, Ben Williams, Marissere, Lyn March Rheumatoid arthritis review article in Best practice 2010 December volume 24;733-745

4.Govindan KS, Basha S, Ramesh V, Kumar CN, Swathi S. A comparative study on serum lipoprotein (a) and lipid profile between rheumatoid arthritis patients and normal subjects. Journal of pharmacy &bioallied sciences. 2015 Apr;7(Suppl 1):S22.

5.Gray S.Firestein, Ralph C.Budd, Edward D.Harris, Iain B.Mcinnes, Shaun Ruddy, John S.Sergent KELLEY'S Textbook of Rheumatology. Eight edition. Vol;1 421-430.

6.Elshereef RR, Darwish A, Ali A, Abdel-kadar M, Hamdy L. Asymptomatic atherosclerosis in Egyptian rheumatoid arthritis patients and its relation to disease activity. International journal of rheumatology. 2015;2015.

7.Plutzky J, Liao KP. Lipids in RA: What do they mean?. Current rheumatology reports.2018 Feb 21;20(2):8.

8. Angeline T, Aruna R, Ramadevi K, Mohan G, Jeyaraj N. Serum lipoprotein (a) and lipid profile in young South Indian patients with myocardial infarction. Indian journal of Clinical biochemistry. 2003 Jan 1;18(1):103-6.

9.Calmarza P, Trejo JM, Lapresta C, Lopez P. Lack of association between carotid intima-media thickness and apolipoprotein (a) isoforms in a sample of Spanish general population. Journal of cardiology. 2013 May 1;61(5):372-7.

10.Giuseppetti GM, Cerioni A, Mangiacotti M, Salaffi F, Carotti M, Grassi W. Atherosclerosis in rheumatoid arthtritis: the role of high-resolution B mode ultrasoundin the measurement of the arterial intima-media thickness. Reumatismo. 2011 Jan 1;59(1):38-49.

11. Carl A. Burtis, Ph.D., Edward R. Ashwood, M.D., David E. Bruns, M.D: Tietz' Textbook of Clinical chemistry and Molecular Diagnostics 5th Edition page : 731-805, 1544-1554, 2132-2188.

12. Tristano AG. Beneficial effects of statins in systemic lupus erythematosus: molecular mechanism involved. Statins: indications and uses, safety and modes of action.:143.

13. Dan L. Longo,DennisL.Kasper, J. Larry Jameson,Anthony S. Fauci,Stephen L. Hauser, Joseph Loscalzo. Harrison's Textbook of internal medicine 18thedition (2012) ; vol 2: page numbers 2136-2149.

14.Van GaalenFA,Toes RE, Ditzel HJ, Schaller M,Breedveld FC, Verweij CL, HuizingaTW. Association of autoantibodies to glucose-6-phosphate isomerase with extraarticular complications in rheumatoid arthritis. Arthritis &Rheumatism: Official journal of the American college of Rheumatology.2004 Feb;50(2):395-9.

15. Dursunoğlu D, Evrengül H, Polat B, Tanrıverdi H, Çobankara V, Kaftan A, Kılıç M. Lp (a) lipoprotein and lipids in patients with rheumatoid arthritis: serum levels and relationship to inflammation. Rheumatology international. 2005 May 1;25(4):241-5.

16. Kumar P, Clark ML. Kumar and Clark's Clinical Medicine E-Book. Elsevier health sciences; 2012 Jun 4.:672-679.

17.Zalewski A, Macphee C. Role of lipoprotein-associated phospholipase A2 in atherosclerosis: biology, epidemiology, and possible therapeutic target. Arteriosclerosis, thrombosis, and vascular biology. 2005 May 1;25(5):923-31.

18.Lourida ES, Georgiadis AN, Papavasiliou EC, Papathanasiou AI, Drosos AA, Tselepis AD. Patients with early rheumatoid arthritis exhibit elevated autoantibody titers against mildly oxidized low-density lipoprotein and exhibit decreased activity of the lipoprotein-associated phospholipase A 2. Arthritis research & therapy. 2007 Feb;9(1):R19.

19.Oei HH, van der Meer IM, Hofman A, Koudstaal PJ, Stijnen T, Breteler MM, Witteman JC. Lipoprotein-associated phospholipase A2 activity is associated with risk of coronary heart disease and ischemic stroke: the Rotterdam Study. Circulation. 2005 Feb 8;111(5):570-5.

20. Bishop ML, Fody EP, Schoeff LE, editors. Clinical chemistry: principles, techniques, and correlations. Lippincott Williams & Wilkins; 2013 Feb 20.

21.McEntegart A, Capell HA, Creran D, Rumley A, Woodward M, Lowe GD. Cardiovascular risk factors, including thrombotic variables, in a population with rheumatoid arthritis. Rheumatology. 2001 Jun 1;40(6):640-4.

22.Wållberg-Jonsson S, Cvetkovic JT, Sundqvist KG, Lefvert AK, Rantapää-Dahlqvist S. Activation of the immune system and inflammatory activity in relation tomarkers of atherothrombotic disease and atherosclerosis in rheumatoid arthritis. The Journal of rheumatology. 2002 May 1;29(5):875-82.

23. Kerekes G, Szekanecz Z, Dér H, Sándor Z, Lakos G, Muszbek L, Csipö I, Sipka S, Seres I, Paragh G, Kappelmayer J. Endothelial dysfunction and atherosclerosis in rheumatoid arthritis: a multiparametric analysis using imaging techniques and laboratory markers of inflammation and autoimmunity. The Journal of rheumatology. 2008 Mar 1;35(3):398-406.

24. Crosby JR, Kaminski WE, Schatteman G, Martin PJ, Raines EW, Seifert RA, et al. Endothelial cells of hematopoietic origin make a significant contribution to adult blood vessel formation. Circ Res 2000; 87:728–30.

25.Champe PC, Harvey RA, Ferrier DR. Biochemistry. Lippincott Williams & Wilkins; 2005.

26. Peters MJ, Van Halm VP, Voskuyl AE, Smulders YM, Boers M, Lems WF, Visser M, Stehouwer CD, Dekker JM, Nijpels G, Heine R. Does rheumatoid arthritis equal diabetes mellitus as an independent risk factor for cardiovascular disease? A prospective study. Arthritis Care & Research: Official Journal of the American College of Rheumatology. 2009 Nov 15;61(11):1571-9.

27.Hill JM, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, Quyyumi AA, Finkel T. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. New England Journal of Medicine. 2003 Feb 13;348(7):593-600.

28.Herbrig K, Haensel S, Oelschlaegel U, Pistrosch F, Foerster S, Passauer J. Endothelial dysfunction in patients with rheumatoid arthritis is associated with a reduced number and impaired function of endothelial progenitor cells. Annals of the rheumatic diseases. 2006 Feb 1;65(2):157-63.

29.Grisar J, Aletaha D, Steiner CW, Kapral T, Steiner S, Seidinger D, Weigel G, Schwarzinger I, Wolozcszuk W, Steiner G, Smolen JS. Depletion of endothelial progenitor cells in the peripheral blood of patients with rheumatoid arthritis. Circulation. 2005 Jan 18;111(2):204-11.

30. Dijk JM, Algra A, Van Der Graaf Y, Grobbee DE, Bots ML. Carotid stiffness and the risk of new vascular events in patients with manifest

cardiovascular disease. The SMART study. European heart journal. 2005 Apr 11;26(12):1213-20.

31.Mäki-Petäjä KM, Hall FC, Booth AD, Wallace SM, Bearcroft PW, Harish S, Furlong A, McEniery CM, Brown J, Wilkinson IB. Rheumatoid arthritis is associatedwith increased aortic pulse-wave velocity, which is reduced by anti–tumor necrosis factor-α therapy. Circulation. 2006 Sep 12;114(11):1185-92.

32. Weber T, O'rourke MF, Lassnig E, Porodko M, Ammer M, Rammer M, Eber B. Pulse waveform characteristics predict cardiovascular events and mortality in patients undergoing coronary angiography. Journal of hypertension. 2010 Apr 1;28(4):797-805

33.Pieringer H, Schumacher s, Stuby U, Biesenbach G. Augmentation index and large-artery remodelling in patients with longstanding rheumatoid arthritis compared with healthy controls. In semiinars in arthritis and rheumatism 2009 Dec 1 (Vol.39, No.3, pp.163-169).

34.Ohtsuka S, Kakihana M, Watanabe H, Sugishita Y. Chronically decreased aortic distensibility causes deterioration of coronary perfusion during increased left ventricular contraction. Journal of the American College of Cardiology. 1994 Nov 1;24(5):1406-14.

35.Kingwell BA, Waddell TK, Medley TL, Cameron JD, Dart AM. Large artery stiffness predicts ischemic threshold in patients with coronary artery disease. Journal of the American College of Cardiology. 2002 Aug 21;40(4):773-9.

36. Arosio E, De Marchi S, Rigoni A, Prior M, Delva P, Lechi A. Forearm haemodynamics, arterial stiffness and microcirculatory reactivity in rheumatoid arthritis. Journal of hypertension. 2007 Jun 1; 25 (6) :1273-8

37.Pasceri V, Yeh ET. A tale of two diseases: atherosclerosis and rheumatoid arthritis. 1999

38. Peters MJ, Symmons DP, McCarey D, Dijkmans BA, Nicola P, Kvien TK, McInnes IB, Haentzschel H, Gonzalez-Gay MA, Provan S, Semb A. EULAR evidence-based recommendations for cardiovascular risk management in patients with rheumatoid arthritis and other forms of inflammatory arthritis. Annals of the rheumatic diseases. 2010 Feb 1;69(2):325-31.

39. Van Doornum S, Brand C, King B, Sundararajan V. Increased case fatality rates following a first acute cardiovascular event in patients with rheumatoid arthritis. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology. 2006 Jul;54(7):2061-8.

40. Haffner SM, Lehto S, Rönnemaa T, Pyörälä K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. New England journal of medicine. 1998 Jul 23;339(4):229-34.

41. Steiner G, Urowitz MB. Lipid profiles in patients with rheumatoid arthritis: mechanisms and the impact of treatment. InSeminars in arthritis and rheumatism 2009 Apr 1 (Vol. 38, No. 5, pp. 372-381). WB Saunders.

42. Schimmel EK, Yazici Y. Increased lipid levels but unchanged atherogenic index in rheumatoid arthritis patients treated with biologic disease modifying antirheumaticdrugs: published experience. Clinical & Experimental Rheumatology. 2009 May 1;27(3):446.

43.Gabay C, McInnes IB, Kavanaugh A, Tuckwell K, Klearman M, Pulley J, Sattar N. Comparison of lipid and lipid-associated cardiovascular risk marker changes after treatment with tocilizumab or adalimumab in patients with rheumatoid arthritis. Annals of the rheumatic diseases. 2016 Oct 1;75(10):1806-12.

44.Danesh J, Collins R, Peto R. Lipoprotein (a) and coronary heart disease: meta-analysis of prospective studies. Circulation. 2000 Sep 5;102(10):1082-5

45. ANGLÉS□CANO ED, DÍaz AD, Loyau S. Inhibition of fibrinolysis by lipoprotein (a). Annals of the New York Academy of Sciences. 2001 Jun 1;936(1):261-75.

46. Ichinose A. Multiple members of the plasminogen-apolipoprotein (a) gene family associated with thrombosis. Biochemistry. 1992 Mar 1;31(12):3113-8.

47.Patthy L, Trexler M, Váli Z, Banyai L, Varadi A. Kringles: modules specialized for protein binding. FEBS letters. 1984 Jun 4;171(1):131-6.

48.Fless GM, ZumMallen ME, Scanu AM. Physicochemical properties of apolipoprotein (a) and lipoprotein (a-) derived from the dissociation of human plasma lipoprotein (a). Journal of Biological Chemistry. 1986 Jul 5;261(19):8712-8.

49. Guevara Jr J, Jan AY, Knapp R, Tulinsky A, Morrisett JD. Comparison of ligand-binding sites of modeled apo [a] kringle-like sequences in human lipoprotein [a]. Arteriosclerosis and thrombosis: a journal of vascular biology. 1993 May;13(5):758-70.

50. Trexler M, Vali Z, Patthy L. Structure of the omega-aminocarboxylic acid-binding sites of human plasminogen. Arginine 70 and aspartic acid 56 are essential for binding of ligand by kringle 4. Journal of Biological Chemistry. 1982 Jul 10;257(13):7401-6.

51.Calmarza P,Trejo JM, Lapresta C, Lopez P. Lack of association between carotid intima -media thickness and apolipoprotein (a) isoforms ina sample of Spanish general population. Journal of cardiology.2013 May 1;61 (5) :352-7

52.Pieringer H, Pichler M. Cardiovascular morbidity and mortality in patients with rheumatoid arthritis: vascular alterations and possible clinical implications. QJM: An International Journal of Medicine. 2010 Nov 10;104(1):13-26.

53. William F Keane, Joanne E Tomassini, David R Neff: Lipid Abnormalities in patients with Chronic Kidney Disease: Implications for the pathophysiology of Atherosclerosis –Review. Journal of Atherosclerosis and Thrombosis, 2013; 20:123-133

54. Erqou S, Kaptoge S, Perry PL, Di AE, Thompson A, White IR, Marcovina SM, Collins R, Thompson SG, Danesh J. Lipoprotein(a) concentration and

the risk of coronary heart disease, stroke, and nonvascular mortality. JAMA 2009;302:412-423.

55. Anna Gries, Institute of Physiology, Medical University of Graz, Austria. Lipoproteins – Role in Health and Diseases;Chapter 32:Lipoprotein (a) – An Overview© 2012 Gries, licensee. InTech. http://dx.doi.org/10.5772/45986

56.Ristić GG, Lepić T, Glišić B, Stanisavljević D, Vojvodić D, Petronijević M, Stefanović D. Rheumatoid arthritis is an independent risk factor for increased carotid intima-media thickness: impact of anti-inflammatory treatment. Rheumatology. 2010 Mar 5;49(6):1076-81.

57. Grover S, Sinha RP, Singh U, Tewari S, Aggarwal A, Misra R. Subclinical atherosclerosis in rheumatoid arthritis in India. The Journal of rheumatology. 2006 Feb 1;33(2):244-7.

58. Burke GL, Evans GW, Riley WA, Sharrett AR, Howard G, Barnes RW, Rosamond W, Crow RS, Rautaharju PM, Heiss G. Arterial wall thickness is associated with prevalent cardiovascular disease in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. Stroke. 1995 Mar;26(3):386-91.

59. De Groot E, Van Leuven SI, Duivenvoorden R, Meuwese MC, Akdim F, Bots ML, Kastelein JJ. Measurement of carotid intima–media thickness to assess progression and regression of atherosclerosis. Nature Reviews Cardiology. 2008 May;5(5):280.

60.Pignoli P. Ultrasound B-mode imaging for arterial wall thickness measurement. Atherosclerosis Rev..1984;12:177-84.

61. Guidelines Committee. 2003 European Society of Hypertension–European Society of Cardiology guidelines for the management of arterial hypertension. Journal of hypertension. 2003 Jun 1;21(6):1011-53.

62. Greenland, P., Alpert, J.S., Beller, G.A., Benjamin, E.J., Budoff, M.J., Fayad, Z.A., Foster, E., Hlatky, M.A., Hodgson, J.M., Kushner, F.G. and Lauer, M.S., 2010. 2010 ACCF/AHA Guideline for Assessment of Cardiovascular Risk in Asymptomatic Adults: Executive Summary: A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines Developed in Collaboration With the American Society of Echocardiography, American Society of Nuclear Cardiology, Society of Atherosclerosis Imaging and Prevention, Society for Cardiovascular Angiography and Interventions, Society of Cardiovascular Computed Tomography, and Society .... *Journal of the American College of Cardiology*, *56*(25), pp.2182-2199.

63. Saigal R, Mathur V, Goyal L. Carotid intima media thickness as a marker of subclinical atherosclerosis in rheumatoid arthritis: a case control study. International Journal of Advances in Medicine. 2016 Dec 24;3(4):942-6.

64.O'leary DH, Polak JF, Kronmal RA, ManolioTA, Burke GL, Wolfson Jr SK. Carotid-artery intima and media thickness as a risk factor for myocardial

infarctionand stroke in older adults. New England Journal of Medicine.1999 Jan 7;340(1):14-22.

65.Qu B, Qu T. Causes of changes in carotid intima media thickness: a literature review. Cardiovascular ultrasound.2015 Dec;13(1):46

66.Park YB,Ahn CW, Choi HK, Lee SH, In BH, Nam CM, Lee SK. Atherosclerosis in rheumatoid arthritis : morphological evidence obtained by carotid ultrasound.Arhritis& Rheumatism: Official journal journal of the American college of Rheumatology.2002 Jul:46(7):1714-9

67.Ristic GG, Lepic T, Glisic B, StanisavljevicD ,Vojvodic D, PetronijevicD.Rheumatoid arthritis is an independent risk factor for increased carotid intima media thickness: impact of anti-inflammatory treatment. Rheumatology.2010 May 5;49(6):1076-91.

68.Giuseppetti GM, Cerioni A, Mangiacotti M, Salaffi F, Carotti M, Grassi W. Atherosclerosis in rheumatoid arthtritis: the role of high-resolution B mode ultrasound in the measurement of the arterial intima-media thickness. Reumatismo. 2011 Jan 1;59(1):38-49.

69.Profumo E, Di Franco M, Buttari B, Masella R, Filesi C, Tosti ME, Scrivo R, Scarno A, Spadaro A, Saso L, Riganò R. Biomarkers of subclinical atherosclerosis in patients with autoimmune disorders. Mediators of inflammation. 2012;2012.

70.Ristić GG, Subota V, Lepić T, Stanisavljević D, Glišić B, Ristić AD, Petronijević M, Stefanović DZ. Subclinical atherosclerosis in patients with

rheumatoid arthritis and low cardiovascular risk: the role of von Willebrand factor activity. PloS one. 2015 Aug 6;10(8):e0130462.

71. Marder W, Khalatbari S, Myles JD, Hench R, Yalavarthi S, Lustig S, Brook R, Kaplan MJ. Interleukin 17 as a novel predictor of vascular function in rheumatoid arthritis. Annals of the rheumatic diseases. 2011 Jun 1:annrheumdis148031.

72.ShahayarS ,Warraich H, McEovyJW, Oni E, Ali SS, Karim A, Jamal O, Blaha MJ, Blumenthal RS, Fialkow J, Cury R. Subclinical cardiovascular disease in plaque psoriasis; association or causal link?. Atherosclerosis. 2014 Jan 1:232(1) :72-8

73. Eder L, Jayakar J, Shanmugarajah S, Thavaneswaran A, Pereira D, Chandran V, Rosen CF, Gladman DD.The burden of carotid artery plaques is higher in patients with psoriatic arthritis compared with those with psoriasis alone. Annals of the rheumatic diseases. 2013 May 1;72(5):715-20.

74.Ahmad HM ,Sarhan EM, Komber U. Higher circulating levels of oxldl% of LDL are associated with subclinical atherosclerosis in female patients with systemic lupus erythematosus. Rheumatology international. 2014 May 1;34 (5): 617-23

75.Missala I, Kassner U, Steinhagen-Thiessen E. A systematic literature review of the association of lipoprotein (a) and autoimmune diseases and atherosclerosis. International journal of rheumatology. 2012;2012.

76. Ishihara KK, Kokubo Y, Yokota C, Hida E, Miyata T, Toyoda K, Matsumoto M, Minematsu K, Miyamoto Y. Effect of plasma fibrinogen, high-sensitive C-reactive protein, and cigarette smoking on carotid atherosclerosis: the Suita study. Journal of Stroke and Cerebrovascular Diseases. 2015 Oct 1;24(10):2385-9.

77.Lorenz MW, von Kegler S, Steinmetz H, Markus HS,Sitzer M. Carotid intima – media thickening indicates a higher vascular risk across a wide age range : prospective data from the Carotid Atherosclerosis progression Study (CAPS) .Stroke. 2006 Jan 1;379(1):87-92

78. Kim MK, Shin J, Kweon SS, Shin DH, Lee YH, Chun BY, Choi BY. Harmful and beneficial relationships between alcohol consumption and subclinical atherosclerosis. Nutrition, Metabolism and Cardiovascular Diseases. 2014 Jul 1;24(7):767-76.

79.Ranadive SM, Yan H ,Lane AD, Kappus RM, Cook MD, Sun P, Harvey I, Ploutz-Synder R, Wooda YA, Wilund KR , Fernhall BO. Aerobic exercise training and arterial changes in African-Americans versus Caucasians. Medicine and science in spots and exercise . 2016 Jan;48(1):90

80.Feairheller DL, Diaz KM, Kashem MA, Thakkar SR, Veerabhadrappa P, Sturgeon KM, Ling C, Williamson ST, Kretzschmar J, Lee H, Grimm H. Effects of Moderate Aerobic Exercise Training on Vascular Health and Blood Pressure in A frican A mericans. The Journal of Clinical Hypertension. 2014 Jul;16(7):504-10.

81. Breton CV, Wang X, Mack WJ, Berhane K, Lopez M, Islam TS, Feng M, Hodis HN, Künzli N, Avol E. Carotid artery intima-media thickness in college students: race/ethnicity matters. Atherosclerosis. 2011 Aug 1;217(2):441-6.

82. Hägg U, Wandt B, Bergström G, Volkmann R, Gan LM. Physical exercise capacity is associated with coronary and peripheral vascular function in healthy young adults. American Journal of Physiology-Heart and Circulatory Physiology. 2005 Oct;289(4):H1627-34.

83.Ayer JG, Harmer JA, Nakhla S, Xuan W, Ng MK, Raitakari OT, Marks GB, Celermajer DS. HDL-cholesterol, blood pressure, and asymmetric dimethylarginine are significantly associated with arterial wall thickness in children. Arteriosclerosis, thrombosis, and vascular biology. 2009 Jun 1;29(6):943-9.

84.Geraci G, Mule G, Costanza G, Costanza G, Mogavero M, Geraci C, CottoneS.Relationship between carotid atherosclerosis and pulse pressure with renal hemodynamics in hypertensive patients. American journal of hypertension. 2015 Aug 1;29(4): 519-27

85. Lee SH, Kim JH, Kang MJ, Lee YA, Yang SW, Shin CH. Implications of nocturnal hypertension in children and adolescents with type 1 diabetes. Diabetes care. 2011 Sep 9:DC_110830.

86. Li Y, Liu J, Wang W, Zhao D. The association between within-visit blood pressure variability and carotid artery atherosclerosis in general population. PLoS One. 2014 May 16;9(5):e97760.

87. Bonek K, Głuszko P. Cardiovascular risk assessment in rheumatoid arthritis–controversies and the new approach. Reumatologia. 2016;54(3):128.

88. Tristano AG. Beneficial effects of statins in systemic lupus erythematosus: molecular mechanism involved. Statins: indications and uses, safety and modes of action.:143.

89.Schimmel EK, Yazici Y. Increased lipid levels but unchanged atherogenic index in rheumatoid arthritis patients treated with biologic disease modifying antirheumatic drugs: published experience. Clinical & Experimental Rheumatology. 2009 May 1;27(3):446.

90.Hage MP, Al-Badri MR, Azar ST. A favorable effect of hydroxychloroquine on glucose and lipid metabolism beyond its anti-inflammatory role. Therapeutic advances in endocrinology and metabolism. 2014 Aug;5(4):77-85.

91.Rantapää-Dahlqvist S, Wållberg-Jonsson S, Dahlen G. Lipoprotein (a), lipids, and lipoproteins in patients with rheumatoid arthritis. Annals of the rheumatic diseases. 1991 Jun;50(6):366.

92.Ehnholm C, Garoff H, Renkonen O, Simons K. Protein and carbohydrate composition of Lp (a) lipoprotein from human plasma. Biochemistry. 1972 Aug;11(17):3229-32.

93.Scanu AM. Lipoprotein (a) as a cardiovascular risk factor. The Journal of Japan Atherosclerosis Society. 1991 Jan 1;19(1):19-21.

PROFORMA

NAME: AGE/SEX:

OPNO:

PROVISIONAL DIAGNOSIS:

OCCUPATION:

RESIDENCE:

HISTORY of Present illness

H/O Early morning joint stiffness (YES/NO)

Duration

Involvement of joints

Monoarticular < 4joint

Name 1. 2. 3. 4.

Polyarticular >5joints

H/O fever (YES/NO)

H/O fatigue (YES/NO)

H/O Malaise (YES/NO)

H/ODepression (YES/NO)

H/O Cachexia (YES/NO)

Initial : Present weight: Muscle fasiculations:

H/O Subcutaneous nodules (YES/NO)

site: size: tenderness:

H/O Dry eye/dry mouth (YES/NO)

H/O Dry Cough/Dyspnea (YES/NO)

H/O Chest pain (YES/ NO)

Remission within 6 months (YES/NO)

PAST HISTORY:

DM HT IHD

Lung disease :

h/o cough, fever, pulmonary tuberculosis and treatment:

Liver disease: H/O jaundice, right hypochondrial pain, abdominal distension:

Thyroid disorder:H/O hoarseness of voice, constipation, intolerance to cold, menstrual irregularities.

Any other illness

DRUG HISTROY:

NSAIDS :

T. Diclofenac, T .Paracetamol,

DMARDS:T.Methotrexate, T.Hydroxy chloroquine, T. Glucocorticoids, T .Prednisolone

Others

PERSONAL HISTORY

Diet

Smoking

Alcohol

Family History

DM	HT	IHD
Lung disease	liver disease	Thyroid disease

Any other illness

GENERAL EXAMINATION:

Anaemia : (YES/NO)

Conjunctiva/nails/tongue- papillae

Jaundice : (YES/NO)

conjunctiva

Generalised lymphadenopathy : (YES/NO)

Number/site/size

Pedal Edema : (YES/NO)

Unilateral /bilateral ;Pitting/non pitting

Nutritional status :

Height: Weight:

VITALS:

PULSE RATE :

Rate:

Rhythm:

Volume:

Site:

BLOOD PRESSURE : mm/Hg

TEMPERATURE:

LOCALEXAMINATION:

Number of the joints involved: name 1. 2. 3.

Joint tenderness

Deformity

Subcutaneous Nodules

SYSTEMIC EXAMINATION:

CARDIOVASCULAR : S1: S2:

MURMUR:

RESPIRATORY : NVBS / Any added sounds

ABDOMEN : Hepatomegaly /Splenomegaly

CENTRAL NERVOUS SYSTEM :

CRANIAL NERVE EXAMINATION:

MOTOR SYSTEM:

SENSORY SYSTEM:

REFLEXS:

PATIENT CONSENT FORM

Name: Age / sex: OP No :

I herewith declare that I have been understood the study (title- "**Association of Lipoprotein (a) and Carotid intima media thickness in Rheumatoid Arthritis patients in prediction of Cardiovascular risk")** in a local language and have fully understood the purpose of this study; methodology, proposed intervention, plausible side effects if any and sequelae.

I have been given the opportunity to discuss my doubts and I have received the appropriate explanation.I understand that my participation in this study is completely voluntary and that they are free to be withdrawn from this study at any point of time without prior notice or without having their medical or legal rights affected.

I permit the author and the research team full access to all their records at any point of time even if I have withdrawn from the study. However their identity will not be revealed to any third party or publication.

I herewith permit the author and the research team to use the results and conclusions arising from this study for any academic purpose, including but not limited to dissertation/thesis or publication or presentation in any level.Therefore in my full conscience give consent to include me in the study and undergo any investigation or any intervention therein.

Patient's sign /Date Investigator's sign

பங்குதாரரின் ஒப்புதல்

பெயர்: வயது/பாலினம்: O.P.No.

நான் இதன்மூலம் உறுதியளிப்பது என்னவெனில் இந்த பரிசோதனைப் பற்றி அனைத்தும் எனக்கு தமிழில் விளக்கப்பட்டது.

இந்த ஆய்வு பற்றி எழுந்த சந்தேகங்களுக்கு எனக்கு விளக்கம் அளிக்கப்பட்டது.

நான் இந்த ஆய்வில் எனது சுயவிருப்பத்துடன் கலந்து கொள்கிறேன் மற்றும் நான் இந்த ஆய்வில் இருந்து எப்பொழுது வேண்டுமானாலும், எந்தவித முன் அறிவிப்பு இன்றி விளகிக்கொள்ளவும் எனக்கு முழு சுதந்திரம் கொடுக்கப்பட்டுள்ளது. மற்றும் இதில் எந்தவிதமான சட்டசிக்கலும் இல்லை என்பதும் எடுத்துரைக்கப்பட்டது.

இந்த பரிசோதனை மூலம் வரும் முடிவுகளை கல்வி சம்பந்தப்பட்ட ஆராய்ச்சிற்காகவும், முதுகலை படிப்பிற்கான ஆராய்ச்சிற்காகவும் இவர்கள் பயன்படுத்திக்கொள்ள நான் முழுமனதுடன் சம்மதிக்கிறேன்.

எனவே நான் எனது மனப்பூர்வமான சம்மதத்துடன் இந்த பரிசோதனையில் பங்கு பெறவும், பரிசோதனை பற்றிய முடிவுகளை இவர்கள் பயன்படுத்திக் கொள்ளவும் சம்மதிக்கிறேன்.

பங்குபெறுபவரின் கையொப்பம் Investigator's கையொப்பம்

தேதி:

ஒப்புதல் கடிதம்

எனது பெயர்:……..

விலாசம்:……

……

……

தலைப்பு: அசோசியேசன் - லைபோபுரோடின் (எ) Lp(a) மற்றும் கரோடிட் இன்டிமா மீடியா அளவு (CIMT)இன் முடக்குவாதம் நோய் (Rheumatoid arteries) உள்ளவர்கள் in கணிப்புகள் (Predication) - இதயநோய் ஆபத்து.

எனதுஉடலில் இருந்து இரத்தம் எடுத்து பரிசோதனை செய்யவும். கரோடிட் குருதி நாளம் அல்டராசோனோகிரபி செய்யவும் நான் மனப்பூர்வமாக சம்மதிக்கிறேன்.

இது குறித்து எதிர்காலத்தில் எந்தவிதமான பிரச்சனையும் நான் எழுப்புதில்லை என உறுதியளித்து இந்த ஒப்புதல் கடித்தில் கையொப்பமிடுகிறேன்.

கையொப்பம்

சாட்சிகள்:

1……

2…….

MASTER CHART FOR CONTROLS

S.No	Age	Sex	T.Chol	TGL	VLDL	LDL	HDL	NonHDL	Lp(a)	CAROTID ARTERY DOPPLER IMT-R	IMT-L	PLAQUES
1	53	F	140	42	8	86	46	94	8	0.4	0.4	-
2	35	F	197	128	26	135	36	161	25	0.4	0.5	-
3	41	F	221	138	28	149	44	177	11	0.5	0.5	-
4	46	F	159	67	13	110	36	123	6	0.4	0.5	-
5	55	F	179	131	26	106	47	132	7	0.3	0.4	-
6	65	F	215	108	22	142	51	164	16	0.2	0.3	-
7	46	F	140	71	14	90	36	104	10	0.3	0.3	-
8	50	F	186	72	14	139	33	153	7	0.4	0.4	-
9	45	M	202	183	37	123	42	160	19	0.4	0.4	-
10	27	F	184	154	31	119	34	150	23	0.3	0.4	-
11	47	F	217	125	25	137	55	162	26	0.4	0.4	-
12	30	F	120	98	20	84	16	104	9	0.6	0.6	-
13	40	F	198	211	42	108	48	150	17	0.4	0.3	-
14	55	F	175	110	22	115	38	137	4.7	0.5	0.4	-
15	52	F	185	92	18	137	30	155	18	0.4	0.5	-
16	49	F	184	115	23	117	44	140	9	0.3	0.3	-
17	39	F	159	102	20	80	59	100	10	0.6	0.4	-
18	52	M	178	91	18	116	44	134	25	0.3	0.5	-
19	38	F	143	69	14	83	46	97	14	0.3	0.3	-
20	29	F	197	129	26	114	57	140	28	0.2	0.3	-
21	32	F	203	178	36	125	42	161	17	0.3	0.4	-
22	49	F	170	179	36	86	48	122	20	0.4	0.3	-
23	47	F	183	114	23	109	51	132	11	0.3	0.4	-
24	57	F	159	94	19	100	40	119	15	0.3	0.4	-
25	46	F	172	152	30	106	36	136	13	0.4	0.5	-
26	33	F	170	115	23	112	35	135	12	0.3	0.3	-
27	40	F	272	168	33	171	65	207	29	0.4	0.6	-

28	37	F	172	154	31	86	55	117	72	0.3	0.5	-
29	42	F	186	136	27	114	45	141	11	0.3	0.3	-
30	56	F	162	125	25	90	47	115	21	0.2	0.3	-
31	44	F	150	141	28	90	32	118	14	0.4	0.5	-
32	37	F	195	70	14	125	56	139	16	0.5	0.4	-
33	41	M	188	68	14	110	64	101	9	0.3	0.3	-
34	51	F	158	109	22	101	35	123	13	0.3	0.4	-
35	39	F	176	97	19	88	69	107	13.6	0.3	0.3	-
36	32	F	196	76	15	137	44	152	14	0.3	0.3	-
37	58	F	214	166	33	119	62	152	23	0.4	0.3	-
38	48	F	110	127	25	48	37	73	8	0.5	0.4	-
39	34	F	208	146	29	111	68	140	21	0.3	0.4	-
40	28	F	179	97	20	103	56	123	19	0.4	0.3	-
41	43	F	182	114	23	120	41	141	33	0.3	0.5	-
42	53	F	138	141	28	71	39	99	5.5	0.2	0.3	-
43	59	F	156	145	29	57	70	86	13	0.4	0.5	-
44	51	F	158	135	27	91	40	118	14	0.3	0.3	-
45	49	F	132	126	26	69	37	95	30	0.6	0.5	+
46	36	F	172	128	26	105	41	131	24	0.5	0.3	-
47	47	M	173	128	24	85	64	109	36	0.3	0.3	-
48	41	F	168	120	25	88	55	113	16	0.4	0.3	-
49	53	F	204	125	25	133	46	158	5.5	0.3	0.4	-
50	58	F	172	132	27	91	54	118	11	0.4	0.3	-

MASTER CHART FOR CASES

S.No	Age (Yrs)	Sex	Duration of RA (Yrs)	T.Chol	TGL	VLDL	LDL	HDL	Non HDL	Lp(a)	CAROTID ARTERY DOPPLER IMT-R	IMT-L	PLAQUES	RA factor
1	52	F	9	196	110	23	130	43	153	59	0.7	0.6	+	+
2	44	F	5	142	80	17	83	42	100	15	0.5	0.5	-	+
3	53	F	4	159	144	33	75	51	108	22	0.4	0.5	-	+
4	50	F	6	157	149	44	66	47	110	14	0.3	0.4	-	+
5	50	F	4	175	77	15	124	36	139	21	0.5	0.5	-	+
6	53	F	15	237	146	29	159	49	188	72	0.8	0.7	-	+
7	53	F	5	220	101	20	143	57	163	23	0.5	0.4	-	+
8	46	F	6	253	85	17	184	52	201	29	0.5	0.5	-	+
9	53	F	4	183	143	35	100	48	135	41	0.7	0.7	+	+
10	29	F	7	181	114	23	120	38	143	36	0.6	0.7	-	+
11	60	F	15	150	87	17	79	54	96	23	0.5	0.5	-	+
12	30	F	11	150	95	19	95	36	114	42	0.5	0.5	-	+
13	46	F	4	175	85	17	111	47	128	18	0.5	0.4	-	+
14	38	F	3	220	180	36	131	53	167	21	0.5	0.5	-	+
15	60	F	3.5	119	82	16	60	40	79	18	0.4	0.3	-	+
16	53	F	15	201	95	19	129	63	138	54	0.8	0.7	+	+
17	30	F	3	213	143	29	139	52	161	16	0.3	0.3	-	
18	40	F	4.7	204	84	17	143	44	160	26	0.5	0.5	-	+
19	34	F	6	157	112	22	117	18	139	27	0.4	0.4	-	+
20	46	F	14	180	67	13	131	36	144	89	0.9	0.8	-	+
21	50	F	15	160	96	10	74	47	113	29	0.5	0.5	+	+
22	53	F	3	193	81	16	132	45	148	41	0.7	0.6	-	+
23	43	F	5	159	75	15	89	55	104	22	0.5	0.5	-	+
24	50	F	2	195	136	27	134	37	158	18	0.5	0.6	-	+
25	33	M	8	139	97	19	93	27	112	17	0.4	0.4	-	+

26	29	F	4	163	69	14	102	47	116	22	0.4	0.5	-	+
27	43	F	3	180	122	24	126	30	150	18	0.5	0.5	-	+
28	53	F	10	201	128	54	154	45	156	44	0.6	0.6	-	+
29	48	F	3	227	118	24	141	62	165	46	0.7	0.6	+	+
30	45	F	3.5	230	111	22	160	48	182	29	0.5	0.5	-	+
31	60	F	10	196	117	19	123	50	146	36	0.6	0.5	+	+
32	44	F	3.6	140	109	22	81	37	103	10	0.3	0.3	-	+
33	44	M	2	165	130	26	87	52	113	44	0.7	0.6	-	+
34	60	F	12	138	52	10	75	52	86	26	0.4	0.4	-	+
35	59	F	15	154	54	11	87	56	98	57	0.6	0.8	-	+
36	49	F	6	169	156	31	87	51	118	20	0.4	0.5	-	+
37	52	F	7	153	85	17	80	56	97	33	0.5	0.5	-	+
38	42	F	5	171	98	20	102	49	122	20	0.3	0.3	-	+
39	38	F	7	144	116	23	80	41	103	34	0.6	0.7	-	+
40	46	F	4	186	126	25	105	56	130	22	0.4	0.4	+	+
41	55	F	10	194	172	34	123	37	157	45	0.7	0.6	-	+
42	38	F	5.7	230	138	28	168	34	191	29	0.5	0.4	-	+
43	49	F	5	164	141	29	87	48	116	22	0.4	0.4	-	+
44	52	F	4	110	91	18	43	49	61	23	0.5	0.5	-	+
45	46	F	3.5	138	94	19	72	47	91	20	0.4	0.3	-	+
46	49	F	11	197	141	28	139	30	167	63	0.8	0.7	+	+
47	49	F	8	210	138	28	141	41	169	55	0.7	0.6	+	+
48	55	M	7	118	168	34	90	64	54	59	0.7	0.7	-	+
49	38	F	3	135	189	38	64	33	102	13	0.3	0.3	-	+
50	59	F	8	196	110	22	135	39	157	41	0.6	0.5	-	+